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INFLUENCE OF THE DOSE OF VIRUS EMPLOYED IN TESTING THE IMMUNITY OF CATTLE VACCINATED AGAINST CATTLE PLAGUE

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THE size of the infecting dose of virus is not normally a matter of importance when considering the immunity of vaccinated beasts to cattle plague. In general field practice, where cattle are vaccinated in order to render them immune to natural infection with the disease, one is considering immunity to a small dose of virus, and when the immunity of vaccinated cattle is tested under laboratory conditions the test dose of virus administered is also relatively small—usually a few drops only of virulent blood. The question whether a very large dose of virus would break down immunity more readily, or at a shorter interval after vaccination, than a small dose would do so, is therefore one that will only arise in special circumstances.

Such special circumstances are at issue when preparing cattle plague antiserum in the Sudan. It has frequently been recorded in earlier publications that it is only in the southern portion of this country that serum can be economically prepared, and that the climatic conditions of the south make it impossible to work for much more than half the year. The country's requirements of serum, moreover, are such that they can be but barely satisfied by working at the highest pressure throughout the season during which operations are possible. The saving of time has therefore as important an influence on the output as any purely technical factor.

In order to save time at the beginning of a working season, prospective serum producing cattle are largely purchased towards the end of the preceding season, but they have to be herded at some distance—local conditions enforce a considerable distance—from the laboratory, in order to conserve the grazing in the immediate vicinity thereof for the next working season. Although infection with cattle plague is

improbable during the storage period, the consequences of its introduction would be so disastrous that all cattle are immunised as soon as possible after purchase. Until two years ago, *i.e.*, before it was known that a single dose of glycerinised vaccine produced an immunity in most individuals of about a year's duration, all had to be immunised by the serum-virus method, which entailed either long journeys to numerous centres at which they were purchased, or driving the cattle to the laboratory from these centres and later distributing them to their wet-season grazing grounds. This laborious procedure has now been superseded by vaccination at the time and place of purchase, but, although this operation has saved a good deal of initial trouble, it has been open to the objection that although immunity in vaccinated cattle is known to be sufficiently sound after several months to withstand an infection test with a small dose of virus—*e.g.*, 1 c.c.—one has not been confident that it would not be broken down by the large doses of virus administered when instituting hyperimmunisation. Pending removal of this uncertainty, the risk of heavy casualties has still entailed some loss of time in immunising the re-collected cattle by the serum-virus method as a preliminary to hyperimmunising them.

The only type of cattle plague vaccine used in the Sudan is the plain glycerinised type, the standard dose being adjusted to represent an average inoculum of 1 gm. of lymphoidal tissue per 100 kg. body weight of beast. The reasons for adopting this vaccine, in preference to the numerous other types that appear to give satisfaction elsewhere, are recorded by the first author in an earlier publication (1936).

In searching the literature for indications of the probable effect of hyperimmunising cattle vaccinated some months earlier with plain glycerinised vaccine, it is not surprising that none was found. It is, however, claimed by Jacotot (1932, 1935) that the immunity of cattle vaccinated with glycerinised vaccine *treated with formaldehyde or toluol* is more readily broken down by a large dose of virus than by a small one. Reference to his actual protocols shows that the evidence on which he bases this conclusion is in both instances extremely slender, since only two or three cattle were used in his experiments. In any case, conclusions drawn from such experiments, whether valid or not, refer only to formalinised and toluenised vaccines, and may not apply to the plain glycernised product.

So far as simple glycerinised vaccine is concerned, one's own (Bennett, 1936) observations would not indicate that a large dose of virus should necessarily be more dangerous to vaccinated cattle than a small one. In the first place it was shown beyond any doubt that the solidity and duration of vaccination-immunity depended almost entirely on qualitative factors in the vaccine and hardly at all on the quantity administered. Furthermore, the virus injected when testing immunity is not a chemical compound incapable of multiplication, but is a living entity, depending for its effects on the degree to which it can invade, and multiply in, the host's tissues. Thus, although on

general immunological grounds it is quite conceivable that an overwhelmingly large dose might break down an immunity that would withstand the exhibition of a small dose, one's previously acquired knowledge of cattle plague immunity would make it doubtful—or at most an open question—whether a few litres of virulent blood would be more likely to do so than a few drops.

The experiments recorded in this paper, which were instituted in order to settle the question, were carried out in two stages. The first series, conducted by the first author in the Khartoum veterinary laboratory, were carried out under the best available conditions, using vaccine of checked initial potency, and cattle which were maintained in isolation, not only from outside cattle but also from each other, throughout the observations. The second series, carried out by the second author at the Malakal serum laboratory, constituted a large scale check, under conditions of routine practice, of the results obtained in Khartoum.

I.—Laboratory Experiments (Khartoum).

The general outline of this series of experiments was as follows :—

(*a*) Prepare a stock of vaccine from the lymphoidal tissues (lymphatic glands, spleen, thymus and tonsils) of several cattle, in order to ensure a representative mixed sample.

(*b*) Vaccinate about 40 young cattle of 300 to 350 lb. live weight with a standard dose, *viz.* : 1 gramme of tissue per 100 kg. body weight.

(*c*) Check the initial immunity by testing a few of the above at an interval of one week after vaccination with 1 c.c. of virulent blood (controls being of course included).

(*d*) At intervals of three, six, nine and twelve months, select eight vaccinated cattle at random, giving six of them 500 c.c. of virulent blood and two of them 1 c.c. only, controls being included in each test.

Very little explanation of the above programme is necessary, but one or two remarks will not be superfluous. There were two reasons for designing the experiment to continue for a maximum period of twelve months. The first was that one would never need to store vaccinated cattle for any longer period before hyperimmunising them (usually one would not need to store them for more than seven or eight months), and the second was that it had already been shown (Bennett, 1936) that, when only given a 1 c.c. test dose of virulent blood, the immunity would, although still very serviceable, show signs of waning at some period short of a year ; there was consequently no point, quite apart from one's immediate objective, in continuing observations for a longer time. Although the primary object was to compare the effects of large and small doses of virus at progressively longer periods after vaccination, arrangement was not made for equal numbers of cattle to be tested throughout the experiment with the two doses. The reason for this was that the behaviour

of vaccinated cattle in the face of a small dose of virus had already been sufficiently worked out up to a period of about ten months, and that therefore only a few beasts, included so as to make the present experiments strictly comparable with the earlier observations, needed to be tested with the small dose up to and including the test at nine months' interval. Fortunately, a few additional cattle which were vaccinated as reserves, in case of casualties from intercurrent disease, were still available when the tests at twelve months' interval were due, and, as will be seen in the ensuing records, these enabled one at this final test not only to employ equal numbers for the small and large doses, but also a slightly larger total number for both. Regarding the " large " dose of virus selected, 500 c.c. of virulent blood for a beast of 300 to 350 lb. live weight is roughly equivalent to what is normally given to routine serum producers as a first hyperimmunising dose (*i.e.*, 1·5 c.c. per 1 lb. body weight).

On 22.3.36, therefore, 40 cattle were vaccinated with a standard dose of fresh vaccine, and seven days later two of these, together with two controls, were given 1 c.c. of virulent blood. Neither vaccinated beast developed any symptoms whatsoever, while both controls died of cattle plague. There was thus no doubt about the initial immunity of the vaccinated herd. The result of the series of three-monthly tests are given in Table I.

TABLE I.

REACTION OF VACCINATED CATTLE TO LARGE AND SMALL DOSES OF VIRUS AT INTERVALS OF 3, 6, 9 AND 12 MONTHS AFTER VACCINATION. DATE OF VACCINATION, 22.3.36.

Interval.	*Virus Dose.*	*Bull No.*	*Symptoms.*	*Result.*
3 months 14.6.36	1 c.c.	2172	Fever 4th to 11th days only	Survived
		2205	No symptoms	„
	500 c.c.	2177	„	„
		2187	„	„
		2193	Fever 8th to 11th days and mild stomatitis	„
		2207	? ? fever	„
		2212	Mild fever 4th to 8th days	„
		2216	„ 4th to 7th days	„
	Controls	2231	Severe cattle plague	Died
		2232	„ „ „	„
		2233	„ „ „	„
6 months 6.10.36	1 c.c.	2202	No symptoms	Survived
		2211	Doubtful fever 5th to 6th days	„
	500 c.c.	2198	Fever 5th to 8th days, a few buccal vesicles 7th day healed by 9th day	Survived
		2204	No symptoms	„
		2206	„	„
		2209	Marked fever 4th to 8th days only	„
		2214	No symptoms	„
		2199	„	„
	Controls	2304	Severe cattle plague	Died
		2305	„ „ „	Survived
		2306	„ „ „	Died

TABLE I—*continued.*

Interval.	*Virus Dose.*	*Bull No.*	*Symptoms.*	*Result.*
9 months 19.12.36	1 c.c.	2197	Fever 5th to 7th days	Survived
		2215	„ 4th to 8th days	„
	500 c.c.	2186	? fever	„
		2188	Fever 3rd to 8th days. Slightly off food 7th to 8th days	„
		2191	Temp. 102·2 morning of 5th and 6th days	„
		2194	„ 102·6 „ 5th day	„
		2203	No symptoms	„
		2213	„	„
	Controls	2376	Severe cattle plague	Died
		2377	„ „ „	„
		2378	„ „ „	„
		2379	No symptoms	Immune
1 year 27.3.37	1 c.c.	2175	Severe cattle plague	Survived
		3179	No symptoms	„
		2184	Fever 4th to 8th days, slightly off food 7th and 8th days	„
		2189	Mild fever 4th to 13th days	„
		2190	No symptoms	„
		2192	Mild fever 4th to 8th days	„
		2195	Fever 6th to 8th days. Marked buccal ulceration 9th to 13th days. Fed well except 11th day	„
	500 c.c.	2174	No symptoms	Survived
		2180	Fever 4th to 10th days. Appreciable buccal ulceration. Fed well	„
		2183	Fever 5th to 8th days	„
		2200	„ 4th to 8th days	„
		2201	No symptoms	„
		2210	Fever 3rd to 9th days (few Theileria)	„
		2218	No symptoms	„
	Controls	2417	Mild cattle plague	„
		2418	Severe cattle plague	Died
		2419	No symptoms	Immune
		2420	Severe cattle plague	Died

The data here tabulated may now be examined with a view to eliciting answers to the two points at issue, namely (i) the purely immunological question whether a large dose of virus is more likely than a small one to break down immunity, and (ii) the practical question whether it will be safe, without further treatment, to hyper-immunise cattle that have been given a single dose of vaccine six or eight months earlier.

The records show without any doubt that the 500 c.c. dose did not, at any interval after vaccination, prove more dangerous than the 1 c.c. dose. At both the third and sixth month it will be observed that one beast tested with 500 c.c. (No. 2193 at the third, and No. 2198 at the sixth) developed mild stomatitis, whereas none of those given 1 c.c. developed more than a febrile reaction. On the other hand,

however, no beast tested at nine months' interval developed more than a thermal reaction, and after twelve months two of the 1 c.c. beasts (Nos. 2175 and 2195) developed quite marked clinical symptoms while only one of the 500 c.c. cattle (No. 2180) did so. Actually the only beast that was so seriously ill as to be in danger of dying was a 1 c.c. beast (No. 2175, which developed all symptoms of advanced cattle plague). In fact, viewing the experiment as a whole, there is complete absence of any evidence that a large dose of virus is more likely than a small one to break down immunity at either a long or a short interval after vaccination.

The question whether, under routine conditions of serum production, it would be safe to hyperimmunise vaccinated beasts after an interval of six or eight months can, in general terms, also obviously be answered in the affirmative. In the foregoing series of tests not a single death occurred, and up to and including the test at nine months' interval not a single vaccinated beast developed more than the very mildest symptoms. So favourable an outcome could not be predicted if a very much larger number of cattle were at issue, as was proved in one's earlier (1936) experiments when one beast out of 38 died when tested with a small dose of virus at ten months' interval. With large numbers, in fact, an odd death or two would not unreasonably be expected, but the laboratory experiments just recorded showed that relatively few casualties would be likely, and a large scale trial would be justified. Such a trial was therefore undertaken, and will now be described.

II.—Field Test (Malakal).

As already indicated, this test was carried out under practical field conditions. Of some hundreds of cattle vaccinated at the time of purchase (June or July, 1937) and left entirely under the supervision of native herdsmen pending the re-opening of the serum laboratory, 69 beasts, selected entirely at random, were hyperimmunised without further treatment several months later. The main bulk of prospective serum producers was not so treated, not so much because heavy casualties were feared (the Khartoum experiments had reassured one on this point) but because it would have been impracticable to keep so large a number of cattle under individual observation.

It had originally been intended to hyperimmunise these cattle by the intramuscular injection of virulent blood at the usual initial rate of 1·5 c.c. per 1 lb. body weight, but in view of the successful outcome of the Khartoum experiments, and in order to test the efficacy of simple vaccination more severely, they were actually injected at the rate of 2 c.c. per 1 lb. Following hyperimmunisation, not only was each beast to be thoroughly examined, but its temperature also recorded, twice daily. The further intention was that all cattle which did not develop marked fever or other physical symptoms of cattle plague were to be bled for serum according to the usual routine (first two bleedings on eighth and eleventh days); cattle showing high

fever or other symptoms of the disease were not to be bled, but were merely to be observed. The hyperimmunising injections were ultimately carried out at intervals varying from 148 to 197 (roughly from five to six and a half months) after vaccination.

In the 69 cattle so treated the observations were as follows :—

(i) Sixty cattle developed no marked fever and no outward symptoms of cattle plague, and were therefore bled for serum according to the usual routine—incidentally with no untoward sequelæ following bleeding.

(ii) Five cattle developed non-fatal plague, in degrees of intensity varying from fever and one or two buccal vesicles to marked stomatitis and some diarrhœa. These were not bled for serum.

(iii) Three cattle died of cattle plague.

(iv) In addition to the above, one beast showed no symptoms before bleeding, but, following bleeding on the eighth day, it developed severe diarrhœa. Whether this diarrhœa was a symptom of cattle plague is uncertain ; in any case the beast was quite normal after the lapse of a few days.

These results of this experiment are not difficult to assess, even though there may be a doubt as to whether the beast described in class (iv) above should not have been included in class (ii). The outcome was not so uniformly successful as in the first set of experiments carried out in Khartoum, but the difference is not statistically significant. Furthermore, it is well known that the cattle of the southern Sudan are less resistant to cattle plague than those of the north. The single unfortunate omission, from the purely scientific standpoint, is that this second experiment at Malakal was not designed to include controls, receiving 1 c.c. of virus, with which to compare the hyperimmunised cattle. The necessity for pressing forward with serum production, however, made it impracticable thus to round off the observations on southern cattle.

From the practical standpoint, especially under Sudan conditions of serum preparation, the indications are nevertheless quite clear : when hyperimmunising large numbers of vaccinated cattle at six months' interval or thereabouts after vaccination, a few casualties are to be expected, but the saving of time effected will be very handsome recompense for the small loss entailed.

Summary.

1. Earlier work, showing that immunity following vaccination with a single dose of (plain glycerinised) cattle plague vaccine persists in most cattle for about a year, is confirmed.

2. The immunity, if artificially tested, is no more likely to be broken down by a large dose of virus than by a small dose.

3. Arising out of the two foregoing conclusions, it follows that cattle destined for use as cattle plague serum producers may be hyperimmunised several months after simple vaccination without fear of incurring more than minimal casualties.

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THREE CASES OF NODULAR NECROSIS (ROECKL'S GRANULOMA) IN THE MUSCLES OF CATTLE.

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I.—Introduction.

This condition was apparently first seen in 1870[1], and later, in 1885, was described in detail by Roeckl[2]. Literature on the subject is scanty, and Verge, Tachon and Drieux[3] in 1937 have only found some twenty references to the disease. Foulerton[4] describes a case in this journal in 1898, and except for a footnote by Young[5] in the English translation of Ostertag's Meat Inspection, no other reference has been observed in English publications. Three interesting cases have come to our notice, in connection with our investigation of " skin lesion " tuberculin reactors[6]. It would seem that the condition must be more common than the amount of literature would indicate, although in view of our observations here reported, it is possible that the development and complete absorption of the lesions may occupy quite a short interval.

The Lesions.

The disease seems peculiar to cattle, except that Cohrs[7] has seen a similar condition in rabbits. Although the muscles are most frequently the sole site of lesions, according to Ziegler[8] the internal organs may also be affected.

Macroscopically the nodules have been described as varying from very small to hazel-nut size, usually intramuscular but occasionally subcutaneous in position. A marked predilection for superficial muscles such as the panniculus, and for the muscles of the tail, has frequently been observed. Section shows a centre of necrotic-looking material of firm consistency and variable colour, with or without foci of calcification, and a well defined fibrous capsule. Histological descriptions include a central zone of necrosis, over which nuclear material is irregularly scattered, and in which small accumulations of round cells and calcified foci may occur. Next a variable zone of loose connective tissue containing leucocytes, plasma cells and pus cells, and finally a dense capsule of fibrous tissue. An abundant invasion of the middle zone with eosinophils is sometimes described. The occurrence of giant cells is disputed, but in other respects descriptions largely agree.

There seems to be no reason to doubt that accounts of Roeckl's Granuloma, of Nodular Necrosis, of Blastomycosis, and some cases of Tuberculosis (Cauchemez[9]) in muscles, are descriptions of the same type of lesion.

II.—Cases seen at the Laboratory.

Case 1.

History.

A Guernsey cow, seven or more calves, in fair condition with good udder and no cough, was found on examination to have a large number of nodules showing under the skin. These lesions (as visible in the living animal) had developed in five months from "very small scarcely noticeable nodules" into very obvious growths, some of which were as large as a tangerine (Fig. 1). The nodules were firm and rounded, not, in the majority of cases, involving the skin, although in a few instances, especially where the skin was naturally tense, there appeared to be some adhesion. No evidence was seen of any external skin lesion. The nodules were very plentiful on the legs, trunk, and neck, and the under side of the proximal third of the tail was packed with growths (Fig. 1). The lesions were painless and cold, and there was no obvious interference with movement of the affected parts.

A reaction was obtained from the double intradermal test with bovine tuberculin, and with Johnin, but not with avian tuberculin.

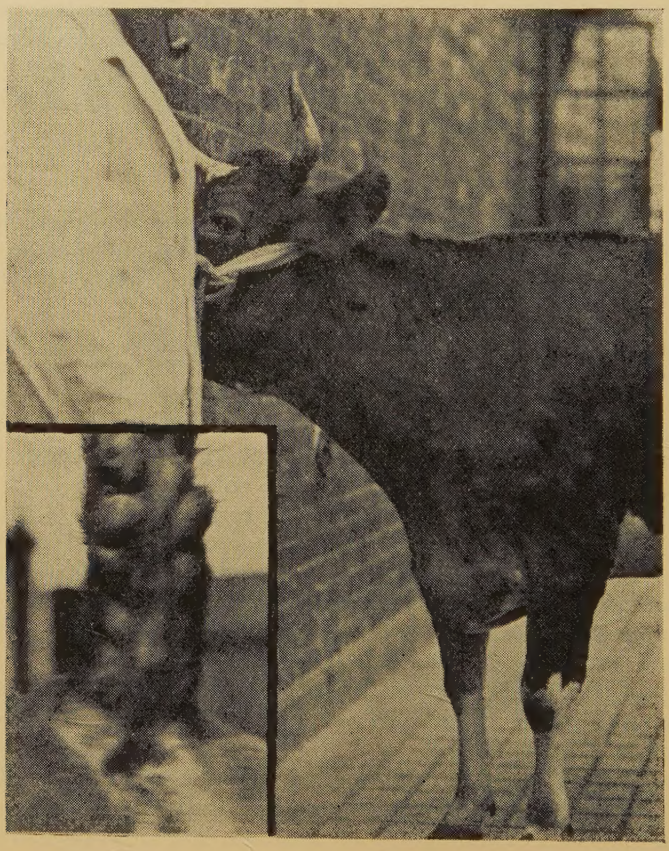

Fig. 1. Case 1. *Inset.* Base of tail.

FIG. 1. Case 1. Skinned left fore leg from shoulder to carpus. Some of the lesions round the shoulder joint have been sectioned horizontally. *Inset.* A sectional portion of muscle. Note the onion-like markings of the lesions. × ½.

A nodule was removed surgically from the neck, it was found to lie, not subcutaneously, as expected, but underneath the sheath of the brachiocephalicus muscle. The animal was finally shot and a *post-mortem* examination carried out.

Post-mortem Observations.

When the carcase was skinned the number of the nodules was seen to be many times greater than visible or palpable in the living animal; the majority of the voluntary skeletal muscles contained one or more lesions.

The heaviest invasion lay in the more superficial muscles, those nodules most prominent during life involving the panniculus, but the deep muscles of the neck and limbs also showed numerous lesions. Nearly one hundred nodules were counted in the musculature of the proximal eighteen inches of the tail, and the lower parts of the limbs were also very severely affected (Fig. 2). In a few cases there was a fibrous adhesion between the connective tissue of the skin and the capsule of the lesion. The nodules were firmly embedded, the great majority lying directly under the muscle sheath, bulging deeply into the muscle as well as distending the sheath outwardly (Fig. 2); a few lesions, particularly those in deep muscles, lay in the depths of the muscle tissue quite distinct from the sheath, and were found only when the tissue was sliced. All nodules were encapsuled by tough fibrous tissue, and section showed a greyish-white sharply demarcated rim enclosing yellow necrotic looking material, with a consistency and colour like Dutch cheese, arranged in finely or coarsely marked concentric rings giving an appearance rather like the cut surface of an onion. In some, but not all cases, a few spots of calcification were present in the centres. Very frequently there appeared to be an outgrowth from the capsule, forming a sort of core from which the rings radiated.

Other Lesions.

The only other lesions noted were (a) tuberculosis of the lungs, mediastinal, bronchial and supramammary lymphatic glands, (b) three coconut-sized and several smaller masses of fat necrosis in the omental fat. No lesions of Johne's disease were seen, and the examination of smear preparations from the bowel mucosae failed to reveal acid-fast organisms.

Case 2.

The data and material of this case are scanty, but the history is interesting. The animal belonged to the same herd as the subject of Case 3.

History.

The animal was a well-bred Guernsey cow which had shown a number of lumps under the skin of the trunk and legs eighteen months before slaughter. These lumps had regressed and finally disappeared, and a month before death the animal showed no visible lesions. The animal had reacted to the tuberculin test, and when it commenced to lose condition was sent for slaughter. Two nodules were received for examination which were "found in the right axilla." Each was the size of a small walnut, and on section showed the typical cut surface and "onion-like" appearance described in Case 1. Strands of muscle tissue were firmly attached to the capsule. No further details of the *post-mortem* examination were received.

Case 3.

History.

The animal was a well-bred Guernsey cow that had calved twice, was in full milk and fair condition and belonged to the same herd as the beast in Case 2. Visible lesions developed and reached their maximum in about six months, when the animal was photographed (Fig. 3). When examined a month later, the nodules appeared to be less prominent, but in other respects resembled those seen in Case No. 1, although much less numerous and not involving the tail. After a further six weeks had elapsed only three lesions were visible, and further regression occurred

FIG. 3. Case 3. First photo.

FIG. 4. Case 3. Second photo, four months later.

until, four months after the original photographs were taken, no lesions could be seen or felt (Fig. 4). (The " roughening " on the flank is caused by coat irregularities, not by lesions.)

The animal had reacted to a double intradermal tuberculin test in the field, and at the laboratory a very severe reaction to bovine tuberculin was followed by a slough the size of a shilling occurring at the site of injection; this can be seen in Fig. 4. No explanation for this unusual reaction is offered; other animals tested at the same time reacted normally.

The animal was losing condition rapidly and commenced to cough; it was obviously tuberculous, and was put down for *post-mortem* examination.

Post-mortem Observations.

Skin, subcutaneous, or intramusuclar lesions were carefully sought, using the photograph as a guide to the most probable sites. One lesion the size of a pea, and several smaller, were found. Some of the nodules, including the largest, were intramuscular in position, but others appeared to have been extruded from the muscle and lay free under the fascia. All were encapsuled by very tough fibrous tissue, with loose foci in the central core which sometimes cut grittily. The characteristic " onion-like " striation was missing. All the lesions found were concerned with the panniculus muscle.

Other Lesions.

There was severe pulmonary tuberculosis, and the hepatic and gastric glands were also involved, being greatly enlarged and caseo-calcareous on section. No lesions were seen in the superficial lymph glands.

III.—Cultural Examinations.

Nodule material from the lesions of Case 1 was ground up into a paste with sterile normal saline, and inoculated on to agar, glucose agar, serum agar, blood agar, glycerine agar, Dorset's egg, Twort's medium, and into peptone broth, glycerine broth, bullocks' heart broth, and gelatine. Batches were incubated under aerobic, anaerobic, and micro-aerophilic (10 per cent. CO_2) conditions. After one month at 37° C. no growth was observed in any tube.

IV.—Animal Inoculations.

Nodule material from the lesions of Case 1, finely ground and suspended in normal saline, was inoculated by intramuscular, subcutaneous, and intravenous or intraperitoneal routes, into fowls, guinea-pigs, and rabbits. When these animals were killed six weeks later, no lesions of any kind were seen, even at the inoculation sites. A heifer also received several intramuscular and subcutaneous injections with the same material. This animal is not yet available for *post-mortem,* but at present, seven weeks after inoculation, it shows no evidence of any lesions.

V.—Histological Examinations.

Smears.

A number of smears were made from the lesions in each case, and stained by the Ziehl-Neelsen method. Examinations failed to reveal any acid-fast organisms.

Sections.

Technique.—Frozen sections were cut and stained with Scharlach R for fat examinations. Paraffin sections were stained (a) by haematoxylin and easin, (b) by iron haematoxylin and Van Gieson, (c) by Stropeni's methyl green-acridine red[10], with a light counterstain of orange G; this was found to be a good differential stain for plasma cells in formol fixed tissues. For organisms the sections were stained by Loeffler's methylene blue, by Gram's method, and by Ziehl-Neelsen. For section work the following modification of Ziehl-Neelsen has been adopted:—Stain first in 1 per cent. watery methyl blue for 10 minutes; pour off, rinse quickly, and stain with hot carbol fuchsin for 10 minutes; decolourise in 5 per cent. HCl in methylated spirit; the red disappears and the blue returns in the acid; rinse as soon as the red is removed, dehydrate and stain lightly in orange G in absolute alcohol. The method gives pretty results, and structural details as well as acid-fast organisms are easily picked out. The preliminary blue staining appears to prevent overstaining of the tissues with carbol fuchsin, and differentiation is more rapid.

The Lesions of Case 1.

Nodules examined under a 2-inch objective showed four clearly marked zones (Fig. 5).

(a) The central zone occupied about four-fifths of the whole, and was arranged in well-defined whorls radiating round a core projecting into it from the outer zones. Centres of calcification were in most cases dotted irregularly over the area, and were most prominent round the core. Under higher powers the whorls were seen to be formed by alternating layers of material rich in nuclei and nuclear debris, and tissue poor in nuclei interwoven with fine strands of connective tissue. The whole zone formed a mass of coagulative necrosis, with karyorrhexis, pyknosis, and calcifying foci. Pyknotic nuclei were sometimes congregated in clumps. The cell outlines were lost, but the substance was bound together by fine fibrils of connective tissue (Fig. 6).

(b) At the edge of the central area there was a sharp transition, without definite boundary, into a narrow zone of foamy cells with a reticulated cytoplasm and supporting leucocytes, small round cells, and a few plasma cells and eosinophils. Connective tissue fibrils from the outer zones passed through into the necrotic centre. The shape and appearance of some of the nuclei in this zone suggested the nuclei of muscle cells, and the remnants of cytoplasm around them showed a fine stippling with fat in frozen sections. The "core" described in (a) was formed largely of this reticular tissue, and some small areas of calcification were present in it with a few giant cells (Fig. 7).

(c) The third zone was formed of fibroblasts and loose connective tissue, in between the fibres of which occurred nests of leucocytes, round cells, a few eosinophils, and numbers of plasma cells.

(d) Zone 4 was a rim of dense fibrous tissue, and appeared to be an extension of the interstitial connective tissue of the muscle bundles.

Zones 3 and 4 were well supplied with vessels, and some blood was present in the reticular zone.

The Lesions of Case 2.

The histology of these sections was essentially the same as seen in Case 1. In neither case were giant cells seen in the central necrotic zone,

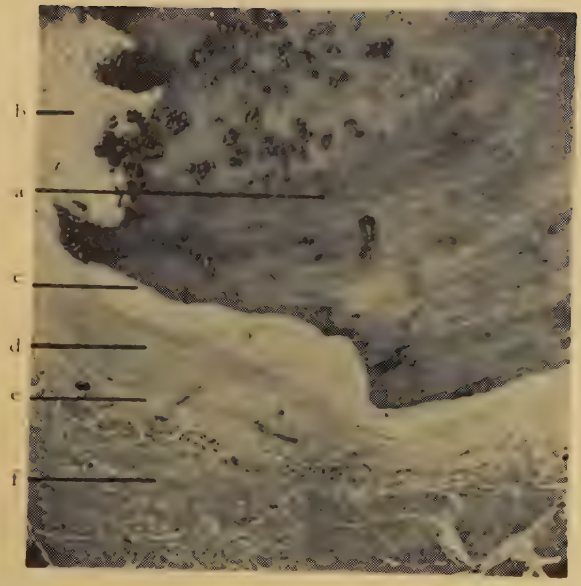

FIG. 5. Nodule from Case 1. × 12. (a) Necrotic zone with whorled appearance and calcified foci. (b) The core. (c) The reticular zone. (d) Loose connective tissue. (e) Capsule. (f) Surrounding muscle.

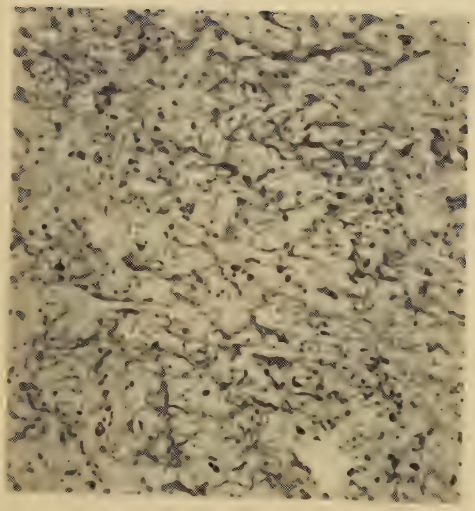

FIG. 6. Necrotic zone with fine strands of connective tissue (dark). Case 1. Van Gieson. × 250.

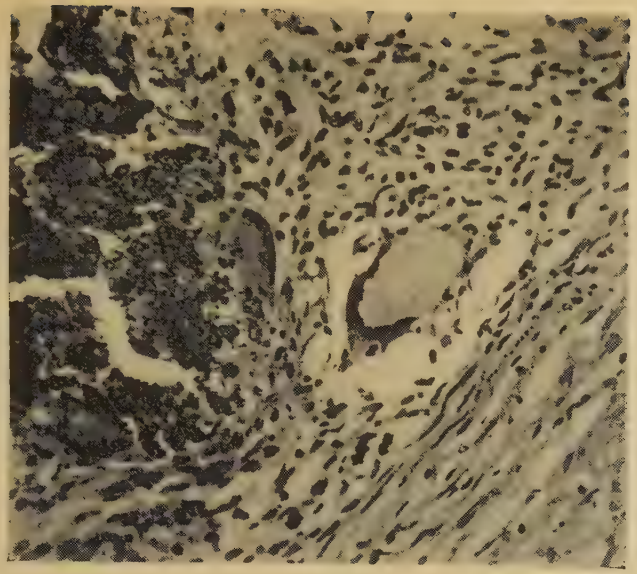

FIG. 7. Giant cells in the reticular zone near a calcified focus. Case 1. H. & E. × 250.

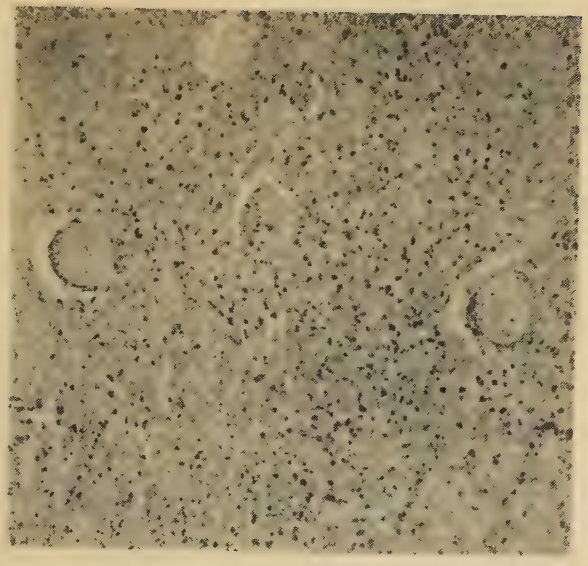

FIG. 8. Degenerating sarcosporidia ? in the necrotic zone. Case 2. H. & E. × 200.

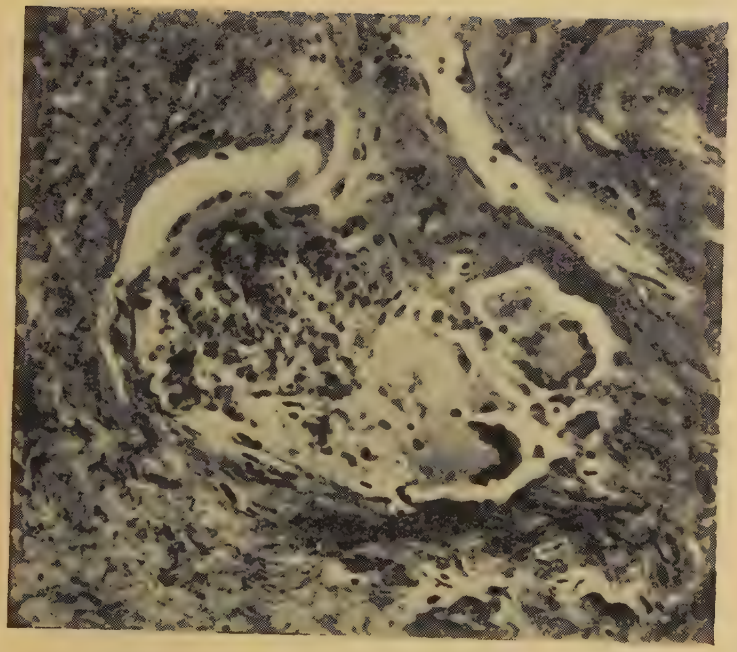

FIG. 9. Island of debris with giant cells. Note the surround of dense fibrous tissue. Case 3. Van Gieson. × 250.

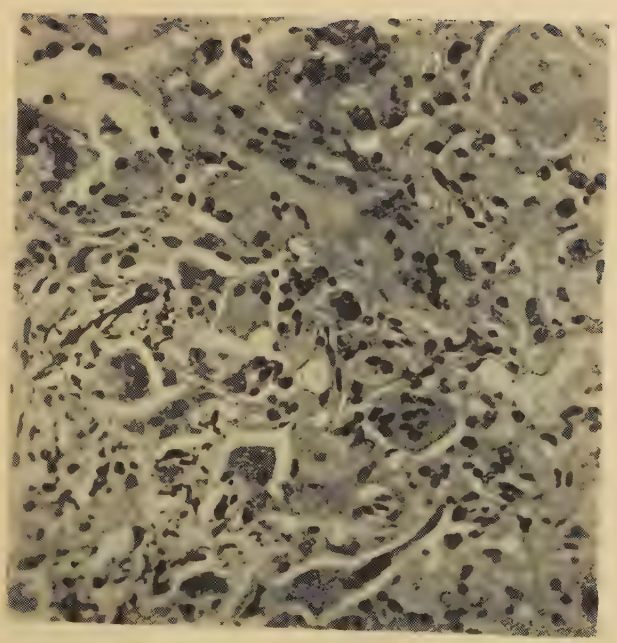

FIG. 10. Portion of another more cellular island, showing ? early giant cell formation. Some minute capillaries are also present but do not show well. Case 3. H. & E. × 250.

but did occasionally occur in the surrounding foamy areas, usually adjacent to centres of calcification and near the "core" of the lesions. In one respect the lesions of Case 2 differed from the rest; sarcosporidia were present in the shreds of attached muscles, and in the necrotic zones there were numerous bodies of about the same size with a capsule enclosing unstained coarsely granular material, most probably degenerating or dead sarcosporidia (Fig. 8). There were present also unstained irregular shaped particles about the size of lymphocytes, suggestive of crystals. These might have been an artefact of preparation or a product of necrosis.

The Lesions of Case 3.

In this case there was some small celled invasion of the interstitial connective tissue of the muscles, and some of the muscle bundles were swollen, intensely stained with eosin and had very flattened shrunken nuclei. In the lesions the "whorled" appearance was lost, and the fibrous tissue in the necrotic central zone had become dense and compact, segregating the remnants of necrotic, calcified, or cellular tissue into islands. These islands contained many irregular giant cells, some of large size, some with peripheral, some with central nuclei, which appeared to be engaged in the removal of the remains of the necrotic tissue (Fig. 9). The connective tissue had brought with it well defined vessels (Fig. 10) which were absent in the corresponding zone in Cases 1 and 2. Nodules of lymphocytes were occasionally present at the edges of the lesions.

Sections stained by Gram's method, Loeffler's methylene blue, and Ziehl-Neelsen failed to reveal the presence of any organisms.

VI.—Discussion.

The three cases described are of considerable interest, Case 1 because of its severity, and Cases 2 and 3 because of the resorption of the lesions. Although the histological details of Case 3 differed somewhat from the others, in view of the analogous histories of Cases 2 and 3 and the situation of the lesions, we are convinced that Case 3 was an advanced stage of resorption of the lesions seen in Cases 1 and 2. It is, however, to be regretted that a nodule was not removed surgically for histological examination, before regression occurred.

Etiology.

The cause of these lesions is not known. Foulerton[4] and others have considered the condition to be a blastomycosis, and Ostertag[11] supports this view; cultural attempts with material from the lesions have however, failed to confirm the presence of the mould. *B. pyogenes* has also been incriminated, and occasionally cultivated from the lesions by Ziegler[8] and others, but whether it is the primary cause or a secondary invader is not clear. The tubercle bacillus, though thought by some workers to be responsible for the lesions, has never been identified, cultured, or obtained from lesion material by animal inoculation, and although tuberculosis is commonly found elsewhere in the affected animals, in view of these negative findings and of the histology of the lesions there seems to be no evidence to support the tubercle theory. Lutter[12], amongst others, thinks that sarcosporidia

are possible etiological agents. Our experiments have afforded negative evidence only; with regard to the sarcosporidia we have described it seems more probable that the parasites were present in the muscle before the lesions were set up, and being engulfed, underwent necrosis with the muscle tissue. We suggest that cases of early infection have never yet been described; all accounts are of healing lesions, the necrotic zones being surrounded by more or less granulation tissue. In view of the possible recovery of the animal without treatment, as seen in Cases 2 and 3, it seems probable that the causative agent may be rapidly removed or destroyed, and until lesions from a very early case are examined by cultural and biological methods, the cause will not be determined. The lesions in all our cases were visible during life, but the time taken to reach the visible stage is not known. Verge, Tachon and Drieux[3] state that the lesions are often not observed until after slaughter for meat. Further evidence in support of this theory of a " fleeting " infection is that although a number of nodules, large and small, were sectioned in each case, the degree of fibrosis and granulation present in different lesions from the same animal was the same. It suggested to us that the various foci had all originated at the same time, and that no spread to fresh foci had occurred from the primary lesions.

Histology.

Previous accounts and our descriptions are in general agreement except regarding the presence of eosinophil leucocytes and giant cells. We have not seen the marked eosinophil invasion of the granulation tissue described by other workers. The presence of giant cells has been denied by more than one author, and Ziegler[8] considers that atrophied muscle cells surrounded by granulation tissue have been mistakenly described as giant cells. In our cases we have no doubt that true giant cells were present, probably only as the lesions get older, but that, if differentiation of giant cell types is permissible, they are more like foreign body giant cells, and they play an active part in the removal of necrotic tissue.

Nomenclature.

What appear to be examples of the same condition have been described as Roeckl's Granuloma, blastomycosis, muscular tuberculosis, and pseudo-tuberculosis. Ostertag[11], who has seen a number of cases, has used the name " Roeckl's Granuloma " in his textbook on Meat Inspection, but Ziegler[8] considers that the lesion is a necrotic one, and that " granuloma " should not be used. Cohrs[7] also prefers the name " Knotige Muskelnekrose " and does not refer to the disease as a granuloma. According to Dorland[13], Virchow defined a granuloma as a " tumour or neoplasm made up of granulation tissue." In our cases, and apparently in others, the bulk of the lesion is necrotic; granulation tissue only comes into the picture when repair is

taking place, and therefore Ziegler's contention seems correct. Our observations of degenerating sarcosporidia suggest to us that the lesion is a focal necrosis of an area of muscle, which becomes encapsulated and attacked by granulation tissue.

Differential Diagnosis.

Tuberculosis of muscle is rare, but, in our cases at least, there seems to be no difficulty in differentiating it from this condition. Macroscopically the " onion-like " cut surface of these lesions is characteristic, although Ziegler[8] states the naked eye appearance is not unlike tuberculosis. However, microscopically the lesions appear quite distinct. The other condition with which confusion might arise is the skin lesion of certain tuberculin reactors that have been reported in the United States and recently in this country[8]. Clinically, a mild case of nodular necrosis is quite easily taken for a severe case of " skin lesions " and it is not easy by palpation, to decide whether the nodules are subcutaneous or intramuscular. " Skin lesions," however, are most frequently found about the legs, a percentage involved the skin itself, and there are nearly always some signs of external ulceration or loss of hair. Nodular necrosis is more commonly visible on the trunk and neck, especially in the panniculus muscle and very typically though not always gives rise to lesions on the under surface of the tail. Ulceration or skin involvement is not observed.

Conclusion.

1. As a result of our investigations it would appear that the lesions of nodular necrosis in the muscles of cattle may undergo absorption.

2. It would seem probable that the condition must occur more frequently than has been reported, but it may be that absorption is a common sequel, and the nearly absorbed lesions would be overlooked in meat inspection. There is no reason to assume that such meat is unfit for human consumption, while obviously affected meat would be unsaleable.

3. We consider the use of the term granuloma is unsatisfactory ; the lesion appears to be originally a necrosis, which is surrounded by chronic inflammatory tissue and repair with fibrosis ensues.

4. Giant cells play a prominent part in the removal of the necrotic tissue. We have not observed the eosinophil invasion described by other workers.

5. No further light is thrown on the etiology. On histological, cultural, and biological grounds it does not appear to be tuberculosis. It seems probable that early lesions have not been described, and that healing has always commenced in those seen. It is possible that the causative agent is dead and when early lesions can be examined culturally and biologically the cause may be found.

Summary.

1. Three cases of nodular necrosis are described in the muscles of cattle.
2. The absorption of such lesions during life is recorded.
3. The morbid anatomy and histology is described and discussed.
4. No further light is thrown on the etiology.

The photograph in Fig. 3 is reproduced by the courtesy of Major Heatley, O.B.E., M.R.C.V.S.

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STUDIES IN ANIMAL TRYPANOSOMIASES.

I.—Nitrogen and Mineral Metabolic Disturbances Induced by *Trypanosoma congolense* and *Trypanosoma brucei*.

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In Tanganyika, as indeed over a very large part of the African continent, the greatest single factor governing stock distribution is the presence of tsetse flies. The cause of this biotic relationship is not the flies but the diseases (trypanosomiases) transmitted by them. Not only because of the need for preventing the impoverishment of cattle-owning tribes, but also because this problem of tsetse flies and trypanosomiases constitutes a serious obstacle in the way of the future economic development of the continent, there has been much work done on animal trypanosomiases. Unfortunately, most of these laboratory studies have been made on small animals and difficulty is often experienced in applying these results to the larger domestic stock. This difficulty arises because there are as many trypanosomiases as there are species of trypanosomes, and *T. congolense*, which is essentially a blood parasite, may produce a totally different syndrome from that of *T. brucei* which may cause death before more than a very few parasites can be found in the blood. The subject becomes even more complicated by the fact that the effects of any one species of trypanosome may vary from one host to another, as for instance the clinical pictures of *T. brucei* in rabbits and rats. In view of these complicating factors it was decided to study two trypanosomiases of major veterinary significance using the larger farm animals and not small laboratory animals as the experimental objects.

There are numerous references in the literature to the effects of nutrition on the course of trypanosome infections. All agree that infected animals become emaciated but certain workers state that this happens even though the appetite remains good. It is generally believed that recovery is possible only on good diets, whilst great importance has been attached to the value of mineral supplements by others. A complete review of the literature is unnecessary here but the following has a direct bearing on the present investigation. Kligler and Geiger (1927-28) found that the removal of any important ration from the diet reduced the survival time of rats infected with *T. evansi*. Henderson *et alia* (1931) described the association of geophagia with trypanosome infections of cattle in Nigeria, and found that the provision of salt and bone-meal together, but not separately, prevented or cured any evidence of pica. Le Roux (1933) stated that bonemeal feeding prevented geophagia in Rhodesian cattle infected with *T. vivax* and *T. congolense*. In order to get precise information as to the effect of different nutritional planes on

the course of trypanosome infections in Tanganyika stock a series of experiments were started. From these results French and Hornby (1934, 1935*a*, *b*) concluded that (a) no improvement of the diet above that of bare maintenance can influence the course of infection in cattle and sheep with *T. congolense* and *T. brucei*, (*b*) improvement of the protein and mineral intake did not influence the course of the diseases, (*c*) after crisis the rate of recovery is correlated with the nutritional plane and (*d*) trypanosome infections do not impair the digestive powers but reduce the food intake and particularly the roughage consumption.

At the same time that these nutritional studies were being made the opportunity was taken of studying the mineral metabolism and the blood chemical changes accompanying the infections. In this paper are collected the results of the changes in the mineral metabolism of the infected animals.

Experimental.

In the nutritional studies nine infected and two control oxen were used for the *T. congolense* experiments together with two infected and two control sheep. In the *T. brucei* experiments three infected and two control sheep were used. Metabolic studies were made on but a few of the animals and the relevant details are given below but reference to the previous publications should be made for more complete details.

The general plan adopted was to make one metabolic trial on the experimental animal and its control before the former was inoculated with trypanosomes. A second and in one case a third trial was carried out at varying intervals after infection, the intervals depending upon the condition of the experimental beasts. The nitrogen and mineral balances set out below were recorded at the same time as the digestibility studies.

Two oxen were fed a sub-maintenance hay ration. Ox 3143 was inoculated with a virulent strain of *T. congolense* and suffered from an acute infection. Crisis was reached in the 20th week when the animal had lost 208 lb. in weight and its red cell count had dropped from 10·0 to 3·6 millions. The second metabolic study was made in the 10th to the 12th weeks, at a time when the anæmia had developed to its maximum, fever was high, and the condition of the animal was that typical of " fly-struck " cattle but had not yet reached the distressing state characteristic of the terminal stages of acute infections.

Two other oxen were fed what was considered to be a maintenance hay ration. Ox 2785 suffered from a chronic infection though inoculated with the same strain of trypanosomes as Ox 3143. Neither the loss of weight, nor the degree of anæmia, nor the condition of the animal was so serious as for Ox 3143. The second metabolic trial was made between the 10th and the 12th weeks after infection when the condition was typical. Crisis was passed in the 27th week and

the animal gradually threw off the infection and attained a state of premunition. A third trial was taken in the long period of body equilibrium which succeeds crisis and precedes premunition.

Two sheep were put on to a sub-maintenance hay ration and the oxen experiment repeated. The infected Sheep W died after an acute infection, the strain of trypanosome injected being the same as was used above for the oxen. The second metabolic study was made between the 8th and 10th weeks when the animal was weak and anæmic but before its appetite was affected. The control animal was sick at the time of the second trial and could not be used for this reason.

Two other sheep were given a maintenance ration of hay and cake, and Sheep M was inoculated with the same trypanosome strain as used above. The resulting infection was acute but not so rapidly fatal as for Sheep W. The second metabolic study was made at the same interval after infection as for Sheep W, when the infected animal was typically " fly-struck," and losing weight.

In the *T. brucei* studies no cattle were examined because this trypanosome exerts a comparatively benign influence on many bovines. Two pairs of sheep were used, one pair received a sub-maintenance hay ration, and the other pair received a productive ration which supplied adequate amounts of energy, proteins, and minerals. On the low ration, Sheep Umoyo died in 7 weeks following an acute infection. The second metabolic trial was made between the 3rd and the 5th weeks after inoculation, when the symptoms of the disease were marked but before there was any serious loss of appetite.

Sheep Tumbo on the good ration also had an acute infection and died in the 12th week after infection was started. The second metabolic study was made from the 3rd to the 5th weeks after infection when the typical syndrome had developed.

The Effect of Trypanosomiases on Nitrogen Balances.

Table I summarises the results obtained in these experiments. Both Oxen 2134 and 3143 were losing weight in their pre-experimental period and this explains their initial negative nitrogen balances. By the time of the second trial the control ox had come into liveweight equilibrium again whereas the infected ox was wasting rapidly. Both Oxen 2785 and 2863 were slightly gaining in weight to begin with but the control ox was in a state of equilibrium by the times of the second and third trials. The infected ox lost weight in the second trial but maintained his weight in the third study. In the sheep studies, with the exception of Sheep Tumbo, increased rates of loss of liveweight were recorded for the infected animals in the second trials.

The figures presented in the table are the average of a ten-day collecting period.

These results indicate quite clearly that there was an increased

TABLE .1.

Average Daily Nitrogen Balances (in gm.N.).

Animal.	Remarks.	Total N. Fed.	Residue N.	Nitrogen Consumed.	Faecal Excretion.	Urinary Excretion.	Total Excretion.	Balance.
Ox 2134.	Pre-experimental.	33.262	-	33.262	14.012	20.995	35.007	- 1.745
Ox 3143.	Period.	33.363	-	33.363	14.590	21.062	35.652	- 2.289
Ox 2134.	Control (after 11 weeks).	38.975	-	38.975	15.690	23.255	38.945	+ 0.030
Ox 3143.	Infected(after 11 weeks).	38.426	-	38.426	15.516	26.388	41.904	- 3.478
Ox 2863.	Pre-experimental	48.976	-	48.976	18.293	21.248	39.541	+ 9.435
Ox 2785.	Period.	48.622	-	48.622	18.189	19.407	37.596	+ 11.026
Ox 2863.	Control (after 11 weeks).	50.957	-	50.957	27.803	19.600	47.403	+ 3.554
Ox 2785.	Infected(after 11 weeks).	51.731	1.494	50.237	27.400	23.636	51.036	- 0.799
Ox 2863.	Control (after 30 weeks).	49.520	-	49.520	29.204	17.111	46.315	+ 3.205
Ox 2785.	Approaching premunition.	49.878	3.092	46.786	25.822	20.091	45.913	+ 0.873
Sheep F.	Pre-experimental	3.260	-	3.260	1.651	1.667	3.318	- 0.058
Sheep W.	Period.	3.260	-	3.260	1.664	1.208	2.872	+ 0.388
Sheep W.	Infected (after 9 weeks).	3.290	-	3.290	1.427	2.545	3.972	- 0.682
Sheep U.	Pre-experimental	17.485	-	17.485	2.287	7.352	9.639	+ 7.846
Sheep M.	Period.	17.485	-	17.485	2.383	7.125	9.508	+ 7.977
Sheep U.	Control (after 9 weeks).	12.627	-	12.627	2.037	6.631	8.668	+ 3.959
Sheep M.	Infected(after 9 weeks).	12.627	-	12.627	1.852	10.950	12.802	- 0.175
Sheep Dunia.	Pre-experimental	5.501	0.221	5.280	2.272	3.311	5.583	- 0.303
Sheep Umoyo.	Period.	5.501	-	5.501	2.283	3.264	5.547	- 0.046
Sheep Dunia.	Control (after 4 weeks).	5.504	-	5.504	2.457	2.500	4.957	+ 0.547
Sheep Umoyo.	Infected(after 4 weeks).	5.504	1.050	4.454	1.490	3.269	4.759	- 0.305
Sheep Majani.	Pre-experimental	10.095	0.147	9.948	3.306	3.893	7.199	+ 2.749
Sheep Tumbo.	Period.	10.095	-	10.095	3.708	3.774	7.482	+ 2.613
Sheep Majani.	Control (after 4 weeks).	10.200	-	10.200	3.663	3.941	7.604	+ 2.596
Sheep Tumbo.	Infected(after 4 weeks).	10.200	0.491	9.709	3.801	4.256	8.057	+ 1.652

Average Daily Calcium Balances (in gm. CaO).

Animal.	Remarks.	Total CaO fed.	Residues CaO.	Total CaO Consumed.	Faecal Excretion.	Urinary Excretion.	Total CaO Excretion.	Balance.
Ox 2134. Ox 3143.	Pre-experimental Period.	13.772 13.822	– –	13.772 13.822	10.560 11.637	0.672 0.670	11.232 12.307	+ 2.540 + 1.515
Ox 2134. Ox 3143.	Control (after 11 weeks). Infected (after 11 weeks).	16.134 15.926	– –	16.134 15.926	14.067 14.925	1.002 0.898	15.069 15.823	+ 1.065 + 0.103
Ox 2863. Ox 2785.	Pre-experimental Period.	20.281 20.135	– –	20.281 20.135	16.968 16.641	0.427 0.589	17.395 17.230	+ 2.886 + 2.905
Ox 2863. Ox 2785.	Control (after 11 weeks). Infected (after 11 weeks).	17.433 17.698	– 0.741	17.433 16.957	16.426 18.385	0.897 0.951	17.323 19.336	+ 0.110 – 2.379
Ox 2863. Ox 2785.	Control (after 30 weeks). Approaching Premunition.	20.790 20.790	– 0.961	20.790 19.829	19.128 17.878	0.885 0.911	20.013 18.789	+ 0.777 + 1.040
Sheep F. Sheep W.	Pre-experimental Period.	1.617 1.617	– –	1.617 1.617	1.071 1.275	0.011 0.028	1.082 1.303	+ 0.535 + 0.314
Sheep W.	Infected (after 9 weeks).	1.637	–	1.637	1.432	0.197	1.629	+ 0.008
Sheep U. Sheep M.	Pre-experimental Period.	1.854 1.854	– –	1.854 1.854	1.456 1.586	0.062 0.058	1.518 1.644	+ 0.336 + 0.210
Sheep U. Sheep M.	Control (after 9 weeks). Infected (after 9 weeks).	1.867 1.867	– –	1.867 1.867	1.564 1.644	0.070 0.068	1.634 1.712	+ 0.233 + 0.155
Sheep Dunia. Sheep Umoyo.	Pre-experimental Period.	1.631 1.631	0.116 –	1.515 1.631	0.870 1.151	0.063 0.040	0.933 1.191	+ 0.582 + 0.440
Sheep Dunia. Sheep Umoyo.	Control (after 4 weeks). Infected (after 4 weeks).	1.645 1.645	– 0.469	1.645 1.176	1.180 1.008	0.045 0.059	1.225 1.067	+ 0.420 + 0.109
Sheep Majani. Sheep Tumbo.	Pre-experimental Period.	8.060 8.060	0.226 –	7.834 8.060	5.572 5.912	0.043 0.041	5.615 5.953	+ 2.219 + 2.107
Sheep Majani. Sheep Tumbo.	Control (after 4 weeks). Infected (after 4 weeks).	8.137 8.137	– 0.971	8.137 7.166	5.393 5.388	0.032 0.032	5.425 5.420	+ 2.712 + 1.746

rate of excretion of nitrogen during the course of infection by either *T. congolense* or *T. brucei*. Further, the results show that this increased excretion was by way of the urine, and may be legitimately attributed to an increased endogenous protein catabolism consequent upon the wasting and fever of the disease, thus bringing trypanosomiases into line with other febrile infections.

The results with Sheep Tumbo indicate that the feeding of a high protein high calorific diet had a protein-sparing action on the wastage of body tissues, whilst in the mild infections on a good diet reported by French and Hornby (1934) no loss of weight took place. In acute febrile infections of man it has been found impossible to maintain nitrogenous equilibrium by this means and it is unlikely that this could be achieved in cases of acute trypanosomiasis.

No complete study was made of the nitrogen partition of the urine, but from the few samples analysed it appeared that both the urea and ammonia excretions were increased. The increased ammonia excretion may indicate an attempt on the part of the body to minimise the loss of body bases that accompanies the acidosis.

The Effect of Trypanosomiases on Calcium Balances.

The calcium studies are summarised in Table II.

Without exception it is seen that there was an increased rate of excretion of calcium during trypanosome infections. In only one case (Ox 2785) was a negative calcium balance recorded, whilst the feeding of lime to Sheep Tumbo allowed calcium storage to continue at a satisfactory rate although its control showed a greater rate of retention. It is easy to see that wherever a deficiency of lime occurs in the ration calcium supplements may be beneficial and even necessary if geophagia is to be prevented, as indeed was reported by Henderson (1931) and Le Roux (1933). In the above studies the rations were sufficiently well-supplied with calcium to prevent any pica symptoms.

The increased excretion of calcium during trypanosomiasis is probably associated with the acidosis of the disease.

The Effects of Trypanosomiases on Magnesium Balances.

The magnesium balances are summarised in Table III.

The significance of these results is not easy to interpret. In some cases the retention of magnesium, after infection has been established, was greater than in the pre-experimental period, but in every case, except that of Sheep M, the rate of excretion was greater for the infected than for the control animals. The changes in the excretion of magnesium produced by the infections were very small, and in spite of the slightly greater rates of elimination it is doubtful if the animal suffers significantly, since retention can still take place on very low intakes.

Average Daily Magnesium Balances. (in gm. MgO).

Animal.	Remarks.	Total MgO Fed.	Residues MgO.	Total MgO Consumed.	Faecal Excretion.	Urinary Excretion.	Total MgO Excretion.	Balance.
Ox 2134.	Pre-experimental Period.	4.306	-	4.306	3.532	0.840	4.372	- 0.066
Ox 3143.		4.321	-	4.321	3.552	0.842	4.394	- 0.073
Ox 2134.	Control (after 11 weeks).	5.047	-	5.047	3.884	0.921	4.805	+ 0.242
Ox 3143.	Infected (after 11 weeks)	4.892	-	4.892	3.891	0.912	4.803	+ 0.089
Ox 2863.	Pre-experimental Period.	6.340	-	6.340	5.614	0.351	5.965	+ 0.375
Ox 2785.		6.295	-	6.295	5.800	0.355	6.155	+ 0.140
Ox 2863.	Control (after 11 weeks).	5.448	-	5.448	4.051	0.428	4.479	+ 0.969
Ox 2785.	Infected (after 11 weeks)	5.530	0.276	5.254	4.492	0.359	4.851	+ 0.403
Ox 2863.	Control (after 30 weeks).	6.003	-	6.003	4.958	0.584	5.542	+ 0.461
Ox 2785.	Approaching Premunition.	6.083	0.540	5.543	4.675	0.556	5.231	+ 0.312
Sheep F.	Pre-experimental Period.	0.421	-	0.421	0.336	0.052	0.388	+ 0.033
Sheep W.		0.421	-	0.421	0.336	0.076	0.412	+ 0.009
Sheep W.	Infected (after 9 weeks).	0.425	-	0.425	0.365	0.087	0.452	- 0.027
Sheep U.	Pre-experimental Period.	0.525	-	0.525	0.558	0.013	0.571	- 0.046
Sheep M.		0.525	-	0.525	0.575	0.011	0.586	- 0.061
Sheep U.	Control (after 9 weeks).	0.531	-	0.531	0.600	0.029	0.629	- 0.098
Sheep M.	Infected (after 9 weeks).	0.531	-	0.531	0.571	0.015	0.586	- 0.055
Sheep Dunia.	Pre-experimental Period.	0.636	0.031	0.605	0.474	0.039	0.513	+ 0.092
Sheep Umoyo.		0.636	-	0.636	0.494	0.055	0.549	+ 0.087
Sheep Dunia.	Control (after 4 weeks).	0.645	-	0.645	0.480	0.066	0.546	+ 0.099
Sheep Umoyo.	Infected (after 4 weeks).	0.645	0.256	0.389	0.359	0.063	0.422	- 0.033
Sheep Majani.	Pre-experimental Period.	1.127	0.038	1.089	0.532	0.038	0.570	+ 0.519
Sheep Tumbo.		1.127	-	1.127	0.505	0.038	0.543	+ 0.584
Sheep Majani.	Control (after 4 weeks).	1.138	-	1.138	0.455	0.017	0.472	+ 0.666
Sheep Tumbo.	Infected (after 4 weeks).	1.138	0.049	1.089	0.567	0.011	0.578	+ 0.511

TABLE IV.

Average Daily Potassium Balances (in gm. K_2O).

Animal.	Remarks.	Total K_2O Fed.	Residues K_2O.	Total K_2O Consumed.	Faecal Excretion.	Urinary Excretion.	Total K_2O Excretion.	Balance.
Ox 2134. Ox 3143.	Pre-experimental Period.	74.796 75.067	- -	74.796 75.067	17.200 17.112	52.803 53.121	70.003 70.233	+ 4.793 + 4.834
Ox 2134. Ox 3143.	Control (after 11 weeks). Infected (after 11 weeks)	87.668 86.426	- -	87.668 86.426	18.791 18.630	51.846 77.892	70.637 96.522	+ 17.031 - 10.096
Ox 2863. Ox 2785.	Pre-experimental Period.	110.145 109.349	- -	110.145 109.349	32.492 31.959	68.183 70.493	100.675 102.452	+ 9.470 + 6.897
Ox 2863. Ox 2785.	Control (after 11 weeks). Infected (after 11 weeks)	94.613 96.060	- 3.936	94.613 92.124	28.653 30.138	63.929 65.551	92.582 95.689	+ 2.031 - 3.565
Ox 2863. Ox 2785.	Control (after 30 weeks). Approaching Premunition.	100.982 102.329	- 7.873	100.982 94.456	23.690 22.799	66.451 64.054	90.141 86.853	+ 10.841 + 7.603
Sheep F. Sheep W.	Pre-experimental Period.	7.317 7.317	- -	7.317 7.317	0.681 0.757	3.518 3.040	4.199 3.797	+ 3.118 + 3.520
Sheep W.	Infected (after 9 weeks).	7.410	-	7.410	0.468	7.030	7.498	- 0.088
Sheep U. Sheep M.	Pre-experimental Period.	8.376 8.376	- -	8.376 8.376	0.860 0.886	6.654 6.384	7.514 7.270	+ 0.862 + 1.106
Sheep U. Sheep M.	Control (after 9 weeks). Infected (after 9 weeks).	8.452 8.452	- -	8.452 8.452	1.159 1.266	6.243 7.241	7.402 8.507	+ 1.050 - 0.055
Sheep Dunia. Sheep Umoyo.	Pre-experimental Period.	6.160 6.160	0.348 -	5.812 6.160	1.589 1.979	4.575 4.484	6.164 6.463	- 0.352 - 0.303
Sheep Dunia. Sheep Umoyo.	Control (after 4 weeks). Infected (after 4 weeks).	6.631 6.631	- 1.480	6.631 5.151	1.712 1.836	4.546 3.643	6.258 5.479	+ 0.373 - 0.328
Sheep Majani. Sheep Tumbo.	Pre-experimental Period.	11.370 11.370	0.346 -	11.024 11.370	3.401 3.189	7.176 6.692	10.577 9.881	+ 0.447 + 1.489
Sheep Majani. Sheep Tumbo.	Control (after 4 weeks). Infected (after 4 weeks).	11.490 11.490	- 0.593	11.490 10.897	3.973 3.889	6.816 8.076	10.789 11.965	+ 0.701 - 1.068

TABLE V.

Average Daily Sodium Balances. (in gm. Na_2O).

Animal.	Remarks.	Total Na_2O Fed.	Residues Na_2O.	Total Na_2O Consumed.	Faecal Excretion.	Urinary Excretion.	Total Na_2O Excretion.	Balance.
Ox 2134.	Pre-experimental Period.	5.244	-	5.244	2.075	3.062	5.137	+ 0.107
Ox 3143.		5.263	-	5.263	2.186	3.053	5.239	+ 0.024
Ox 2134.	Control (after 11 weeks).	6.155	-	6.155	2.516	3.930	6.446	- 0.291
Ox 3143.	Infected (after 11 weeks)	6.059	-	6.059	2.716	4.571	7.287	- 1.228
Ox 2863.	Pre-experimental Period.	7.722	-	7.722	0.874	6.531	7.305	+ 0.417
Ox 2785.		7.666	-	7.666	0.838	6.187	7.025	+ 0.641
Ox 2863.	Control (after 11 weeks).	6.663	-	6.663	1.332	5.332	6.664	- 0.001
Ox 2785.	Infected (after 11 weeks)	6.764	0.192	6.572	1.178	7.178	8.356	- 1.784
Ox 2863.	Control (after 30 weeks).	6.275	-	6.275	1.169	5.145	6.314	- 0.039
Ox 2785.	Approaching Premunition.	6.358	0.440	5.918	1.011	5.009	6.020	- 0.102
Sheep F.	Pre-experimental Period.	0.513	-	0.513	0.351	0.560	0.911	- 0.398
Sheep W.		0.513	-	0.513	0.274	0.434	0.708	- 0.195
Sheep W.	Infected (after 9 weeks).	0.520	-	0.520	0.211	0.818	1.029	- 0.509
Sheep U.	Pre-experimental Period.	0.904	-	0.904	0.326	0.448	0.774	+ 0.130
Sheep M.		0.904	-	0.904	0.339	0.475	0.814	+ 0.090
Sheep U.	Control (after 9 weeks).	0.906	-	0.906	0.380	0.731	1.111	- 0.205
Sheep M.	Infected (after 9 weeks).	0.906	-	0.906	0.298	0.610	0.908	- 0.002
Sheep Dunia.	Pre-experimental Period.	0.551	0.028	0.523	0.192	0.430	0.622	- 0.099
Sheep Umoyo.		0.551	-	0.551	0.189	0.395	0.584	- 0.033
Sheep Dunia.	Control (after 4 weeks).	0.558	-	0.558	0.101	0.301	0.402	+ 0.156
Sheep Umoyo.	Infected (after 4 weeks).	0.558	0.115	0.443	0.102	0.389	0.491	- 0.048
Sheep Majani.	Pre-experimental Period.	2.636	0.029	2.607	0.632	1.654	2.286	+ 0.321
Sheep Tumbo.		2.636	-	2.636	0.588	1.899	2.487	+ 0.149
Sheep Majani.	Control (after 4 weeks).	2.660	-	2.660	0.590	1.251	1.841	+ 0.819
Sheep Tumbo.	Infected (after 4 weeks).	2.660	0.415	2.245	0.648	1.204	1.852	+ 0.393

TABLE VI.

Average Daily Chlorine Balances (in gm. Cl).

Animal.	Remarks.	Total Cl Fed.	Residues Cl.	Total Cl Consumed.	Faecal Excretion.	Urinary Excretion.	Total Cl Excretion.	Balance.
Ox 2134. Ox 3143.	Pre-experimental Period.	10.157 10.194	- -	10.157 10.194	0.924 0.945	8.736 8.736	9.660 9.681	+ 0.497 + 0.513
Ox 2134. Ox 3143.	Control (after 11 weeks). Infected (after 11 weeks)	11.905 11.736	- -	11.905 11.736	1.055 1.059	9.166 9.171	10.221 10.230	+ 1.684 + 1.506
Ox 2863. Ox 2785.	Pre-experimental Period.	15.769 14.849	- -	15.769 14.849	1.288 1.742	12.527 11.106	13.815 12.848	+ 1.954 + 2.001
Ox 2863. Ox 2785.	Control (after 11 weeks). Infected (after 11 weeks)	12.865 13.060	- 0.353	12.865 12.707	2.640 1.864	8.265 9.751	10.905 11.615	+ 1.960 + 1.092
Ox 2863. Ox 2785.	Control (after 30 weeks). Approaching Premunition.	11.587 11.742	- 0.781	11.587 10.961	1.725 1.195	8.719 8.799	10.444 9.994	+ 1.143 + 0.967
Sheep F. Sheep W.	Pre-experimental Period.	0.994 0.994	- -	0.994 0.994	0.096 0.072	0.681 0.536	0.777 0.608	+ 0.217 + 0.386
Sheep W.	Infected (after 9 weeks).	1.006	-	1.006	0.075	0.985	1.060	- 0.054
Sheep U. Sheep M.	Pre-experimental Period.	1.041 1.041	- -	1.041 1.041	0.070 0.073	1.087 0.949	1.157 1.022	- 0.116 + 0.019
Sheep U. Sheep M.	Control (after 9 weeks). Infected (after 9 weeks).	1.053 1.053	- -	1.053 1.053	0.094 0.090	0.931 0.928	1.025 1.018	+ 0.028 + 0.035
Sheep Dunia. Sheep Umoyo.	Pre-experimental Period.	1.571 1.571	0.035 -	1.536 1.571	0.015 0.015	1.537 1.169	1.552 1.184	- 0.016 + 0.387
Sheep Dunia. Sheep Umoyo.	Control (after 4 weeks.). Infected (after 4 weeks).	1.244 1.244	- 0.181	1.244 1.063	0.017 0.034	1.227 1.110	1.244 1.144	+ - 0.000 - 0.081
Sheep Majani. Sheep Tumbo.	Pre-experimental Period.	4.628 4.628	0.030 -	4.598 4.628	0.059 0.040	4.878 4.466	4.937 4.506	- 0.339 + 0.122
Sheep Majani. Sheep Tumbo.	Control (after 4 weeks). Infected (after 4 weeks).	4.673 4.673	- 0.603	4.673 4.070	0.142 0.047	2.647 2.163	2.789 2.210	+ 1.884 + 1.860

The Effect of Trypanosomiases on Potassium Balances.

The data collected on the potassium balances is presented in Table IV.

These figures show that not only was the rate of excretion of potassium increased during infection but also that the infected animals were in a state of negative balance. The increased output of potassium was *via* the kidneys.

Since there is a marked loss of weight during trypanosomiasis it is only natural to infer that the increased excretion of potassium is partly due to the elimination of the potassium contained in the autolysed tissues.

The Effect of Trypanosomiases on Sodium and Chlorine Balances.

The variations in the sodium balances caused by the trypanosomes are shown in Table V.

In all the *T. congolense* experiments the rate of excretion of sodium was increased during the infection, and all infected animals were in a state of negative sodium balance. In the *T. brucei* experiments, the infected sheep on the low sodium intake maintained his rate of sodium elimination whereas his control showed an increased retention rate. With the infected sheep on a high salt ration, retention of sodium occurred during the infection but this was at a lower rate than in the control animal.

The chlorine balances are shown in Table VI.

Marked differences are to be observed in the changes produced in the chlorine balances by trypanosome infections. With Ox 2785 and Sheep W and Dunia an increased rate of excretion was recorded. Ox 3143 and Sheep M and Tumbo showed an increased rate of retention, although for Ox 3143 and Sheep Tumbo the rate of excretion was greater than for their controls. The cases Ox 3143 and Sheep M appear to be somewhat anomalous in that an increased chlorine retention accompanies an increased excretion of sodium and potassium. Of course this may be due partly to the animals excreting buffer or organic acids instead of chlorides at the time of these trials.

In a number of human febrile infectious diseases it is known that on salt-poor diets increased excretions of sodium and chlorine are obtained, but that on salt-rich diets a retention of these elements takes place. If the two anomalous cases of Ox 3143 and Sheep M are not considered, it is seen that the changes in animal trypanosomiases are similar. With the rations low in sodium and chlorine fed to Ox 2785, Sheep W and Sheep Dunia an increased rate of excretion of sodium and chlorine followed infection, but with Sheep Tumbo on a high salt-containing ration a storage of these elements was recorded.

The Effect of Trypanosomiases on Phosphate Balances.

Table VII gives the details of the phosphate balances.

TABLE VII.

Average Daily Phosphate Balances (in gm. P_2O_5).

Animal.	Remarks.	Total P_2O_5 Fed.	Residues P_2O_5.	Total P_2O_5 Consumed.	Faecal Excretion.	Urinary Excretion.	Total P_2O_5 Excretion.	Balance.
Ox 2134. Ox 3143.	Pre-experimental Period.	7.645 7.673	- -	7.645 7.673	6.125 6.709	0.421 0.406	6.546 7.115	+ 1.099 + 0.558
Ox 2134. Ox 3143.	Control (after 11 weeks). Infected (after 11 weeks).	8.970 8.834	- -	8.970 8.834	6.875 7.988	0.664 0.760	7.539 8.748	+ 1.431 + 0.086
Ox 2863. Ox 2785.	Pre-experimental Period.	11.258 11.177	- -	11.258 11.177	6.945 6.809	0.531 0.590	7.476 7.399	+ 3.782 + 3.778
Ox 2863. Ox 2785.	Control (after 11 weeks). Infected (after 11 weeks).	9.680 9.827	- 0.339	9.680 9.488	7.141 10.322	0.663 0.535	7.804 10.857	+ 1.876 - 1.369
Ox 2863. Ox 2785.	Control (after 30 weeks). Approaching Premunition.	10.249 10.386	- 0.140	10.249 10.246	8.333 7.874	0.611 0.454	8.944 8.328	+ 1.305 + 1.918
Sheep F. Sheep W.	Pre-experimental Period.	0.748 0.748	- -	0.748 0.748	0.899 0.806	0.007 0.008	0.906 0.814	- 0.158 - 0.066
Sheep W.	Infected (after 9 weeks).	0.757	-	0.757	1.097	0.057	1.154	- 0.397
Sheep U. Sheep M.	Pre-experimental Period.	1.205 1.205	- -	1.205 1.205	0.837 0.878	0.081 0.079	0.918 0.957	+ 0.287 + 0.248
Sheep U. Sheep M.	Control (after 9 weeks). Infected (after 9 weeks).	1.218 1.218	- -	1.218 1.218	0.937 0.893	0.054 0.174	0.991 1.067	+ 0.227 + 0.151
Sheep Dunia. Sheep Umoyo.	Pre-experimental Period.	0.665 0.665	0.028 -	0.637 0.665	0.537 0.591	0.038 0.029	0.575 0.620	+ 0.062 + 0.045
Sheep Dunia. Sheep Umoyo.	Control (after 4 weeks). Infected (after 4 weeks).	0.673 0.673	- 0.119	0.673 0.554	0.559 0.467	0.025 0.018	0.584 0.485	+ 0.089 + 0.069
Sheep Majani. Sheep Tumbo.	Pre-experimental Period.	0.820 0.820	0.015 -	0.805 0.820	0.409 0.548	0.048 0.043	0.457 0.591	+ 0.348 + 0.229
Sheep Majani. Sheep Tumbo.	Control (after 4 weeks). Infected (after 4 weeks).	0.829 0.829	- 0.116	0.829 0.713	0.600 0.603	0.043 0.033	0.643 0.636	+ 0.186 + 0.077

Except for Sheep Dunia all infected animals showed an increased rate of excretion of phosphate, and with Sheep Dunia the rate of excretion was greater than for its control.

Discussion.

The above results indicate that *T. congolense* and *T. brucei*, although of markedly different pathogenicity, both cause an increased excretion of nitrogen, body bases, chlorides and phosphates during their infection of cattle and sheep. The work of French and Hornby (1934, 1935*a*, *b*) has shown that the feeding of protein and mineral supplements does not influence the course of the resulting infection provided that the rations are above that of bare maintenance. On the other hand, the work of Henderson (1931) and Le Roux (1933) has shown that when cattle are subsisting on rations poor or deficient in minerals then geophagia develops with the trypanosome infection. The results of the present series confirm the results of these workers, and explain why it was that bone-meal and salt were the supplements found necessary to cure the geophagia.

Unpublished work indicates that in the larger farm livestock trypanosome infections lead to production of lactic acid in the body and a lowering of the alkali reserve. The excretion of body bases in an attempt to get rid of this acidosis is but a natural consequence, and it is easy to see that once a sufficiency of minerals in the ration is provided, any increased supply cannot influence the course of the infection.

Summary.

T. congolense and *T. brucei* infections in cattle and sheep cause an increased excretion of nitrogen, body bases, chlorides and phosphates.

It is with great pleasure that I record my thanks and indebtedness to Mr. H. E. Hornby for help and advice whilst carrying out the above work.

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STUDIES IN ANIMAL TRYPANOSOMIASES.

II.—Disturbances Produced in the Plasma Proteins by *Trypanosoma congolense* and *Trypanosoma brucei*.

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In certain trypanosomiases of the larger farm animals, the infected beast may suffer a serious loss of weight, become debilitated and anæmic without ever showing trypanosomes in blood smears. Diagnosis from clinical symptoms is complicated by the fact that the syndrome of chronic trypanosomiasis closely resembles and is often reinforced by those produced by worm infestations, over-work and under-nutrition. As a result efforts have been made to evolve a serological test which would confirm the results of symptomatic diagnoses. Except in special circumstances no such test has been found satisfactory, but the results of investigations with the formol-gel, mercuric chloride, and aldehyde tests, etc., indicate that marked disturbances occur in the serum protein fractions during trypanosomiasis.

The first reference concerning the plasma protein changes during trypanosomiasis appears to be that of Mayers (1905) who found that in *T. brucei* infection in a dog the albumin/globulin ratio was diminished. Sice *et alia* (1931) recorded this same lowering of the albumin/globulin ratio in cases of human trypanosomiasis, and Trensz and Jardon (1933) made a similar observation when working with *T. berberum* infections of guinea-pigs and rabbits. The latter also recorded an increase in the euglobulin fraction. Horgan and Bennett (1929) working on *T. soudanense* infections of camels found that the euglobulin increased during infection at the expense of the pseudoglobulin fraction. In view of these observations it was decided that the variations in the plasma protein fractions should form part of the biochemical studies made alongside the initial nutritional investigations on trypanosome-infected farm stock (French and Hornby, 1934, 1935*a*, *b*). These changes form the substance of this paper, which deals with the variations caused by *T. congolense* in cattle, sheep, and a donkey, and by *T. brucei* in sheep, a donkey and guinea-pigs.

Experimental.

For full details of the larger animals, planes of nutrition, conditions of management and details of the infections, previous papers (French and Hornby, 1934, 1935*a*, *b*) should be consulted. It is sufficient, however, for the purpose of this article to give the following brief summaries of the cases.

Oxen 2134 and 3143 were placed on a sub-maintenance hay ration and Ox 3143 infected with *T. congolense*. An acute infection resulted,

and the animal became so weak and anæmic that he collapsed in the 20th week.

He was brought round and from then onwards never showed another parasite in blood smears. The ration was improved at this time by the addition of cotton-seed, and the condition of the experimental ox improved steadily until he was discharged in the 57th week after inoculation.

Oxen 2863, 2785 and 2855 were put on to a maintenance hay ration ; No. 2863 serving as the control ox. Ox 2785 suffered from a chronic *T. congolense* infection and crisis occurred about the 27th week. Body weight then remained constant for the next 19 weeks, during which time the trypanosomes gradually disappeared from blood smears. A state of premunition was reached and the ox had regained his condition by the time he was discharged in the 57th week after inoculation. Ox 2855 suffered from an acute infection although inoculated with the above strain of *T. congolense*. Crisis was reached in the 12th week, but in spite of an improved ration and treatment with antimosan the animal died in the 16th week after inoculation.

Oxen 2386 and 3730 were allowed an *ad lib.* hay ration and both were infected with the above strain of *T. congolense*. Ox 3730 suffered from an acute infection and died after nine weeks. The condition of Ox 2386 became so critical in the 20th week that he had to be treated with antimony to save his life. The ration was improved from the 40th week and the ox was discharged as recovered in the 52nd week.

Oxen 3046, 2225 and 2864 were given cotton-seed daily in addition to an *ad lib.* hay ration. Oxen 3046 and 2225 were infected with a mild strain of *T. congolense* and suffered from a very mild infection, which did not cause loss of liveweight. Crisis was considered to have occurred respectively in the 15th and 18th weeks after inoculation. Both animals recovered without drug treatment and were discharged in the 49th week. Ox 2864 was not given this mild strain of *T. congolense* but the same strain as was used for the previous cases. An acute infection resulted and the ox was treated in the 9th week to save his life. He then made a good recovery, to be discharged in the 31st week after inoculation.

Ox 3622 was given a good mixed ration and infected with the above virulent strain of *T. congolense*. An acute infection resulted and the ox died in the 10th week.

Sheep W and M were inoculated with the above virulent strain of *T. congolense*, but whilst Sheep W received a sub-maintenance hay ration, Sheep M was given a maintenance ration of hay and cake. In both cases the infection was acute and death took place in the 12th and 18th weeks respectively.

Sheep Umoyo, Imani and Dunia were placed on a poor ration of hay and the two former inoculated with a virulent strain of *T. brucei*. Acute infections resulted and death took place in the 6th week in both cases.

Sheep Majani and Tumbo were given a good mixed ration of hay and concentrates and the latter was infected with the above *T. brucei* strain. After an acute infection death took place 12 weeks later.

Donkeys 22, 17 and 3 were put on to an *ad lib.* hay ration, No. 22 serving as the control. Donkey 3 was inoculated with *T. brucei* and died after an acute infection in the 5th week. Donkey 17 was inoculated with *T. congolense* but showed very few clinical symptoms. However, in the 8th week after inoculation, *T. brucei* suddenly appeared and then the animal rapidly went down to death in the 11th week.

Six guinea-pigs referred to in Table IX were all given the same diet. Guinea-pigs 3, 4, 5 and 6 were inoculated with a *T. brucei* strain capable of producing death in 18 to 21 days. These animals showed " swarming " infections when they were killed 18 days after inoculation.

In all these cases the trypanosomiasis blood was drawn at intervals into oxalate and then centrifuged. The plasma protein fractions were estimated by a micro-Kjeldahl method after using the Na_2SO_4 precipitation procedure of Howe (1921). The results of these analyses are collected into Tables I to IX.

Discussion.

The results of this series of experiments agree with those of other workers by showing that a profound change takes place in the proportional distribution of the plasma protein fractions during trypanosomiasis. This is true for both *T. brucei* and *T. congolense* infections of cattle, sheep, donkeys, and guinea-pigs, and further the disturbance is not influenced by the nutrition of the infected animal.

The total protein content of the plasma does not change in any constant manner as a result of trypanosome infections or the accompanying anæmia. In the *T. congolense* infected oxen the three fatal cases of Oxen 3730, 2855 and 3622 showed a decrease in the total protein content, whilst this value also fell until crisis in the acute infection of Ox 2864. Ox 2386 on the other hand showed a steady increase until crisis. In the infections of Oxen 3143, 2785, 3046 and 2225 an initial drop took place but by the time of crisis the total protein had returned to a value higher than its initial level. The two sheep fatally infected with *T. congolense* showed a steady decrease of the total protein value until death. In the *T. brucei* infections Sheep Umoyo and Tumbo showed a greater decrease in their total protein contents than did their controls, whilst Sheep Imani showed practically no change in this value. Donkeys 3 and 17 both showed decreases after infection, but in the guinea-pigs the infections did not appear to have had any influence on the protein level of the plasma. These inconstant variations in the total protein of the plasma agree with the results of Sice *et alia* (1931) when working on *T. gambiense* infections of man, of Krijgsman (1934) when working with

T. evansi infections in rats, and of Launoy and Lagodsky (1937) when studying *T. annamense* infections of rabbits.

The non-protein nitrogen (N.P.N.) was higher than normal at crisis or death in most cases examined. Ox 2855 showed no increase of N.P.N. but the last analysis was made three weeks before death. In the chronic *T. congolense* infection of Ox 2785 no increase of this value was recorded around crisis. No increase was noted in the bloods of the infected guinea-pigs but it is uncertain how far off death from trypanosomiasis these animals were when they were killed. Ox 2863 showed no increase up to crisis but as soon as this animal and Ox 2864 were treated with antimosan an increase of the N.P.N. value occurred. This increase of plasma N.P.N. following antimosan treatment resembles the results of Weiss and Corson (1922) following arsphenamine injections. From the above results it appears that the plasma N.P.N. is not affected until the last stages of fatal infections or until crisis is reached in non-fatal cases.

This agrees with the findings of Linton (1930) for *T. equiperdum* in rats, Randall (1934) for *T. evansi* in horses, and Launoy and Lagodsky (1937) for *T. annamense* in rabbits.

The proportional amount of fibrinogen is not altered by these trypanosome infections.

In every case of infection, irrespective of the trypanosome species or of the kind of host, the albumin proportions decreased up to crisis or up to death. In those cases that recovered, whether or not therapeutic aid was given, the albumin proportions returned to their normal values in from five to eight weeks after crisis.

With the decrease in the proportional amount of the albumin fraction as the disease progressed a corresponding increase took place in the proportional amounts of total globulin. These results agree with earlier workers that the albumin/globulin ratio is decreased during trypanosomiasis. The relative proportions of the pseudo-globulin fraction change but slightly, in most cases the changes parallel those of the albumin fraction but are less in extent. In a few cases no change or even an increase of pseudo-globulin was recorded. The greatest change in the relative proportions of the protein fractions occurs in the euglobulin. With the decrease of the albumin proportions the euglobulin percentage increases, a maximum value being recorded at crisis or at the time of death in fatal infections.

After crisis the proportional amount of euglobulin gradually decreases to its normal value or to a value slightly greater than its initial figure. The time taken for the euglobulin proportions to return to normal is longer than in the case of the albumin and is not complete until 10 to 12 weeks after crisis or treatment. In the case of Ox 2785 in which a long period (19 weeks) of liveweight equilibrium succeeded crisis, during which the trypanosomes gradually disappeared from blood slides before the ox reached a state of premunition, the euglobulin proportions returned to normal much more slowly than

in animals which had received drug treatment or which recovered spontaneously from a mild infection.

In the *T. brucei* infections of sheep and donkeys the increase of the euglobulin and the decrease of the albumin proportions were greater in extent than in the *T. congolense* infections of cattle, sheep and donkeys. It appears also that in acute infections of *T. congolense* in cattle the changes are of a greater order than in cases of infection with mild strains of trypanosome.

Trensz and Jardon (1933) recorded an increase in the euglobulin fraction of the plasma proteins during *T. berberum* infections of rabbits and guinea-pigs, similarly Horgan and Bennett (1929) reported an increase of euglobulin in *T. soudanense* infections of camels, but unlike the results of the present series these latter workers found that the euglobulin proportional increase took place chiefly at the expense of the pseudoglobulin fraction and that the albumin was less affected than in this study.

Lloyd and Paul (1928-29) working on kala-azar, a disease of man caused by another genus of the Trypanosomidiae, found that protein changes of the same type as those in this series occurred during infection. Also they found a similar difference in the rate of return to normal of the euglobulin and albumin fractions following antimony treatment.

Summary.

1. Marked disturbances occur in the plasma protein proportional distribution during infection of cattle, sheep, donkeys and guinea-pigs with *Trypanosoma congolense* and *Trypanosoma brucei*. Details are given of the compositions of normal blood and the changes produced during the different infections on varying nutritional levels.

2. The total plasma protein content did not vary in any constant manner.

3. The non-protein nitrogen showed an increase only at crisis or near death. An increase in N.P.N. followed antimosan injections.

4. The proportional amount of fibrinogen is not influenced by trypanosome infections.

5. The relative proportions of the albumin fraction decrease during infection, reaching a minimum value at death or crisis. During recovery from crisis the albumin percentage returns to normal in from five to eight weeks.

6. With the decrease of albumin, the proportional amounts of total globulin increase. This increase is due to the great increase in the relative proportions of euglobulin. With the passing of crisis the euglobulin percentage returns to normal in from 10 to 12 weeks.

7. The plasma protein changes are greater in *T. brucei* than in *T. congolense* infections. Similarly the changes are greater in acute than in mild cases of trypanosomiasis.

I wish to record my thanks to Mr. H. E. Hornby, Director of Veterinary Services, not only for permission to carry out this work and to publish the results, but also for advice and help.

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STUDIES IN ANIMAL TRYPANOSOMIASES.

III.—The Effects of *Trypanosoma congolense* and *Trypanosoma brucei* on Blood Urea.

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At the time when this series of experiments was initiated, only one reference in the literature was known concerning the effect of trypanosomiasis on blood urea levels. Jones (1933) examined the bloods of cattle and sheep in Uganda and though a decrease was found in several cases of *T. congolense* infections in only one case was an increase of blood urea recorded. High values were found for the non-protein nitrogen and uric acid in the blood by Linton (1930) in the terminal stages of fatal infections of rats by *T. equiperdum*, whilst Randall (1934) recorded an increase of non-protein nitrogen and creatinine at crisis in *T. evansi* infections of horses. Both workers attributed these findings to impairment of the renal excretory function following injury to the kidneys by the parasites.

Graf (1933), working with other protozoan infections of bovines, found that the blood urea was increased in fatal and severe cases of anaplasmosis (*A. marginale* infection). Similarly in the single case of piroplasmosis (*B. bigemina* infection) examined he found an increase of blood urea.

It was therefore decided to obtain further information on the level of blood urea during trypanosome infections of large animals at the time when other biochemical studies were being made (French, 1937*a*) and the results obtained are presented below. Since the completion of these experiments Launoy and Lagodsky (1936, 1937) have published their observations during *T. annamense* infection of rabbits. They found that in the first few weeks of infection the blood non-protein nitrogen and urea did not show any marked variations from normal. In the last stages of the disease, when the animals were dying, increases of blood urea were observed in certain cases.

Experimental.

The details of the infections have been given in earlier publications by French and Hornby (1934 and 1935*a*, *b*) together with the systems of rationing and observations on the anæmia and clinical symptoms of the diseases.

The blood urea was estimated by the Van Slyke aeration method as described by Cole (1926). The figures for control and infected cattle and sheep are given in Tables I to III.

TABLE I.

BLOOD UREA-NITROGEN VALUES DURING INFECTION OF CATTLE WITH *T. congolense* (MGM./100 ML.).

Weeks after Inoculation.	*Poor Hay Ration.*		*Maintenance Hay Ration.*		*Good Ration of Hay and Cotton Seed.*		Ad lib. *Hay Ration.*	
	Ox 3143 (*Infected*).	*Ox 2134* (*Control*).	*Ox 2785* (*Infected*).	*Ox 2863* (*Control*).	*Ox 3046* (*Infected*).	*Ox 2225* (*Infected*).	*Ox 2386* (*Infected*).	*Ox 3730* (*Infected*).
0	18·7	16·1	17·0	17·0	18·1	18·1	17·3	19·5
2	15·9	—	17·5	—	17·8	18·8	—	—
4	17·3	18·8	20·8	18·8	17·1	18·1	14·7	16·8
6	19·6	—	18·9	—	17·8	16·4	9·9	14·3
8	16·8	19·1	18·4	19·6	—	—	—	—
9	—	—	—	—	—	—	—	15·8
10	17·8	—	19·1	—	15·8	17·5	14·4	Died 3 days later.
13	19·4	20·1	19·8	20·1	—	—	16·0	
15	14·4	—	16·8	—	—> 15·6	15·6	12·8	
19	16·6	16·6	15·9	16·6	15·6	—> 16·6	15·8	
20	—> 15·5	17·9	—	—	—	—	—> 17·2	
22	18·8	—	17·2	—	17·3	18·1	—	
25	16·5	15·8	17·7	16·5	16·5	16·0	18·4	
31	15·9	16·3	—> 16·8	17·1	15·9	16·3	15·7	
35	16·3	17·2	17·5	18·3	15·1	16·2	15·3	
40	16·5	18·1	18·1	16·9	Discharged.		16·1	
44	17·6	15·6	16·9	17·5			Discharged.	
	Discharged.		Discharged.					

Note. —> Indicates where Crisis occurred.

TABLE II.

BLOOD UREA-NITROGEN VALUES DURING INFECTION OF SHEEP WITH *T. congolense* (MGM./100 ML.).

Weeks after Inoculation.	*Good Hay and Cake Ration.*	*Poor Hay Ration.*
	Sheep M (Infected).	*Sheep W (Infected).*
0	14·3	12·8
2	10·4	10·4
4	14·6	14·5
6	16·1	16·1
8	15·8	16·3
11	15·8	14·8
14	15·6	Died 7 days later.
17	14·9	
	Died 9 days later.	

TABLE III.

BLOOD UREA-NITROGEN VALUES DURING INFECTION OF SHEEP WITH *T. brucei* (MGM./100 ML.).

	Good Ration.		*Maintenance Hay Ration.*		
Weeks after Inoculation.	*Sheep Tumbo (Infected).*	*Sheep Majani (Control).*	*Sheep Umoyo (Infected).*	*Sheep Dunia (Control).*	*Sheep Imani (Infected).*
0	13·4	12·6	14·3	8·8	11·6
3	14·9	13·1	17·9	11·6	9·9
6	17·9	15·4	22·6	14·8	14·5
9	15·2	11·6	Died 1 day later.	—	Died the same day.
	Died 12 days later.	—	—	—	—

The blood urea values fluctuate even in the healthy controls, the variations being presumably controlled by the volume of liquid consumed, and the rate of urine excretion. In the infected animals the variations are no greater than for the controls, with the single exception of Sheep Umoyo. In this case there was but a slight increase in the blood urea the day before death took place, and it is not certain that this rise is significant.

DISCUSSION.

These results support the findings of Jones (1933) and of Launoy and Lagodsky that there is no increase of blood urea during trypanosome infections except at the end of the disease when the host is moribund. It is doubtful, if this terminal increase can be attributed specifically to the infection, because it is more likely induced by the increased protein catabolism of the general autolysis of body tissue.

Previous results (French, 1937*a*) have shown that these animals were losing body proteins during the infections. This would tend to

cause an increase of blood urea, but since there is no evidence that this occurs in cattle and sheep it can be assumed that neither *T. congolense* nor *T. brucei* damages the kidney tissues sufficiently to cause impairment of renal functions.

SUMMARY.

There is no alteration of the blood urea content as a result of infection of cattle and sheep by *Trypanosoma congolense* and *Trypanosoma brucei*.

I am grateful to Mr. H. E. Hornby for the facilities for doing this work and for permission to publish the results.

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EPIZOOTIC ADENOMATOSIS IN THE LUNGS OF SHEEP—COMPARISONS WITH JAAGSIEKTE, VERMINOUS PNEUMONIA AND PROGRESSIVE PNEUMONIA.

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During the past two years a lung disease of sheep has been spreading increasingly in Iceland, causing great losses to the farmers, many of whom have lost between 50 and 80 per cent. of their flocks. Investigations began in the autumn of 1936, when, at the request of the Icelandic Government, two of us (N. D. and G. G.) began to make an enquiry into its nature and origin.

Preliminary remarks on sheep farming in Iceland.—The management of sheep in Iceland is very different from what it is in Great Britain and on the Continent. During the winter they are housed and stall-fed over a period varying between one and four months according to the severity of the weather. In the spring they graze the pastures in the proximity of the farms but in the latter part of June are driven into the mountain pastures in the interior of the country where they remain until the middle of September. At that time they are rounded up—a procedure taking several days—and gradually driven towards the large collecting folds which are situated at the heads of the main valleys, and are capable of holding up to 4,000 sheep. Here the sheep are closely penned together for two or three days while being separated according to earmarks into the flocks belonging to individual farmers, and are afterwards driven away to the farms.

As will be pointed out later these great collections appear to play an important part in the spreading of the disease from one farm to another, and the crowding of the sheep closely together during the winter in small badly lighted houses with practically no special provision for ventilation, appears to be almost ideal for the dissemination of an infectious respiratory disease.

Origin of the Disease.—The spread of the disease in question can usually be traced without any difficulty from one farm to another, and sometimes from one sheep to another, as will be shown later. Tracing backwards along the course of the epidemic one is led to a certain farm, Deildartunga, in Borgarfjörour (in the south-west part of Iceland) where, all the evidence seems to suggest, the disease originated. To this farm a year-old karakul ram was imported from Germany in 1933, arriving at Deildartunga at the end of December, 1933, and was immediately put in use to serve the ewes. Afterwards it was kept in a separate compartment with one ewe and her lamb,

which was born in the previous autumn, these three being kept there together throughout the winter. No disease was noticed in the ram during this period, but in the spring, when the flock was driven into the mountains, the ram was seen to be dyspnœic and in poor condition and as it did not return from the mountains in the autumn it is thought that it probably died there from the disease. The ewe died at the end of May, 1934, soon after lambing, without any attention being paid to the cause of death. The lamb, however, which lived until the autumn, 1934, was noticed then to be dyspnœic and was killed. About the same time two other sheep died following many days' illness, and from that point onwards losses increased rapidly in the flock, although practically no lambs died until the spring of 1935. From this time onwards, however, losses steadily continued without intermission, so that between April 21st and December 17th, 1935, the farmer had lost 259 ewes out of 475. The greatest loss occurred during one week of the autumn, 1935, causing the farmer finally to decide to send all the remaining 390 lambs to the slaughterhouse. Deaths still continued among the adult sheep, however, and the stock has now been reduced to about 15 per cent. of what it was.

Similar losses have now been experienced on numerous other farms and it has become the general practice on infected farms to slaughter all lambs in the autumn instead of only a certain proportion, experience having taught the farmers that it is useless to try to keep them, a large proportion being invariably lost as a result of the disease.

From the centre at Deildartunga the disease has spread excentrically into the neighbourhood, and has now attacked the sheep in approximately one-third of the whole country.

Clinical Symptoms.—The disease is very insidious in its onset and runs a chronic course so that sheep may have been affected for weeks before the presence of adenomatosis is suspected even by an experienced observer. Indeed, if the disease has not reached an advanced stage a flock at rest, containing many affected sheep, may be looked at most carefully without any sickness being noticed.

The first symptom to appear is an occasional cough, slight at first but later spasmodic and prolonged as if the sheep were trying to get rid of exudate from the bronchi. Respiration becomes more or less accelerated. The expression of the face grows duller, the sheep is less active than normally, and if driven for a long distance has a tendency to lag behind the flock, and once getting out of breath takes one or two hours before the respiration returns to normal. At no time, however, unless a secondary infection intervenes, is there any elevation of temperature.

Comparatively early during the course of the disease auscultation reveals moist râles somewhere in the lungs, especially in the lower parts. This is so noticeable that where the disease has reached an advanced stage, the breathing causes a characteristic sound which may be heard by anyone standing in the midst of the affected flock, the multiple râles resembling the sound of slowly boiling porridge.

If the râles are not heard in a resting flock they may appear after the flock has been driven a short distance.

As the disease advances, respiration tends to become more frequent, and sooner or later, sometimes in an early stage, the most characteristic symptom of the disease will appear, *viz*, the increased bronchial secretion.

At first this secretion is only slightly increased, being just sufficient to keep the nostrils glistening and moist. Later it will be noticed as a thin, frothy mucus, which pours out of the nostrils when the sheep inclines its head towards the ground. This is regarded as almost a pathognomonic symptom. The amount of the secretion may reach extraordinary proportions, as much as 155 c.c. having been collected in one instance by holding the sheep head downwards in the morning. To be able to collect between 10 and 60 c.c. of exudate when an affected sheep is held up in this way is a common occurrence, and it is probable that in some instances the exudation of this fluid exceeds 500 c.c. during the course of 24 hours. That the secretion comes from the lungs and not from the nose, is easily seen at slaughtering, when great amounts of frothy mucus will flow out of the trachea.

The duration of the disease after the first appearance of symptoms is very variable, but may on the average be reckoned at about two or three months. Sometimes the sheep may die one or two weeks after the onset of symptoms, although such a rapid course is rare. On the other hand the disease may run a course of three to six months and even as long as a year. There is no loss of appetite and but little loss in condition until the latest stage, when the sheep are apt to become very emaciated. Recovery is extremely rare, although undoubtedly occurring; most farmers assert that no affected sheep will recover. Probably the chance of recovery is less than 5 per cent.

Not infrequently the temperature rises some few days before death, but this always indicates the onset of terminal pneumonia due to some secondary infection. Sudden death without pneumonia is not rare, however, especially when sick sheep are driven or in some other way submitted to muscular strain, as a result of which they may suddenly fall down dead. As a rule, however, the sheep are not given the chance to die of disease, being slaughtered as soon as the first symptoms are noticed. Another factor, which seems to be of great importance in enhancing the disease, is chill, a sudden drop in the temperature always being followed by an increase in the death rate, which may be very considerable.

Morbid Anatomy.—The pathological changes are practically restricted to the lungs and lower respiratory passages, there being no apparent change in the upper respiratory tract. The exudate already referred to is found in more or less large amounts but as a rule there are no conspicuous changes in the mucosa, which is usually of a clean, white colour, without any ulcers.

In uncomplicated cases, when the sheep has been killed before reaching the terminal stage, the lungs are found considerably enlarged,

and sometimes enormously so (Fig. 1). They are of a light grey or light purplish colour, with more or less widespread greyish patches (Fig. 2), which usually do not protrude on the surface. This greyish tissue, which is very characteristic of the disease, may first be seen as a small nodule of the size of a cherry or larger, not quite distinctly limited and not protruding from the surface, so differing from the worm nodules. On the cut surface this greyish tissue is seen to present numerous small nodules, whitish grey in colour and slightly elevated, measuring from 1 mm. up to 1 cm. in diameter, and of irregular outline. In the first stages normal lung substance exists between the nodules, but as the disease advances more and more nodules appear, until they are seen lying packed closely together. Around the affected area greyish translucent nodules may be seen dispersed on the cut surface about 1 mm. in diameter, or even smaller, yet easily distinguishable from the yellowish worm nodules associated with infestations of *Muellerius capillaris*, which is found in almost every sheep's lung in Iceland. As the disease advances the infiltrated area increases, and at the same time other parts of the lungs are affected, particularly the lower parts of both lungs and the anterior (apical) lobe of the right lung. The marginal spread is often conspicuous, both lungs being affected along their lower borders. On the surface the tissue is of a greyish or a slightly purplish hue, the pleura being sometimes slightly thickened, and slight adhesions are not rare in long standing cases.

This affected tissue has a very different consistence from hepatised, inflamed lung tissue. When pressed between two fingers it is found to be friable, and very easily broken down, in many respects it resembles a soft, cancerous growth. It is frequently waterclogged, and when a severely affected lung is left overnight in a container, a considerable amount of water will be seen to have flowed out of it. The cut surface does not give the ordinary impression of pulmonary œdema, however, as a frothy liquid does not exude from it in any considerable quantity. A frothy mucus is seen to flow from all of the bronchi on the slightest pressure. The increase in water content appears to be limited to the affected area as the rest of the lung is usually normal. The enlargement of the lung is not only dependent upon the decrease of elasticity but also to an actual increase in size of the affected parts, which occurs to an extraordinary extent, as is often very strikingly seen when the cardial lobe of the left lung and the apical lobe of the right lung are involved. These lobes may then reach three to four times their normal size. When an affected lung is compared with a healthy one it will be seen that the enlargement is chiefly a result of the increase in size of the affected tissue although the unaffected parts may also be enlarged. It may be observed, however, that the apparently healthy tissue is not entirely devoid of change, for small nodules will usually be noticed on close observation in the vicinity of the affected parts, growing sparser with increasing distance from the principal focus.

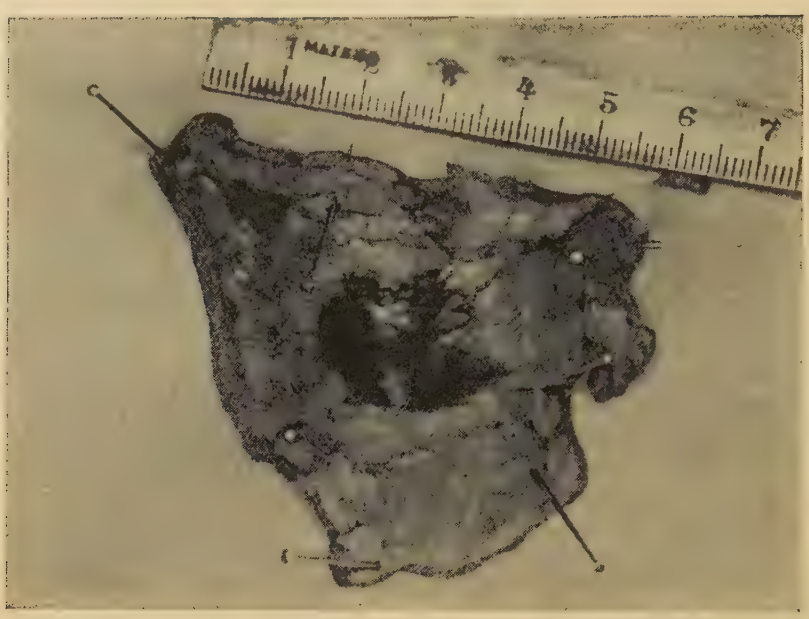

FIG. 1. Beginning nodule as seen from the surface.

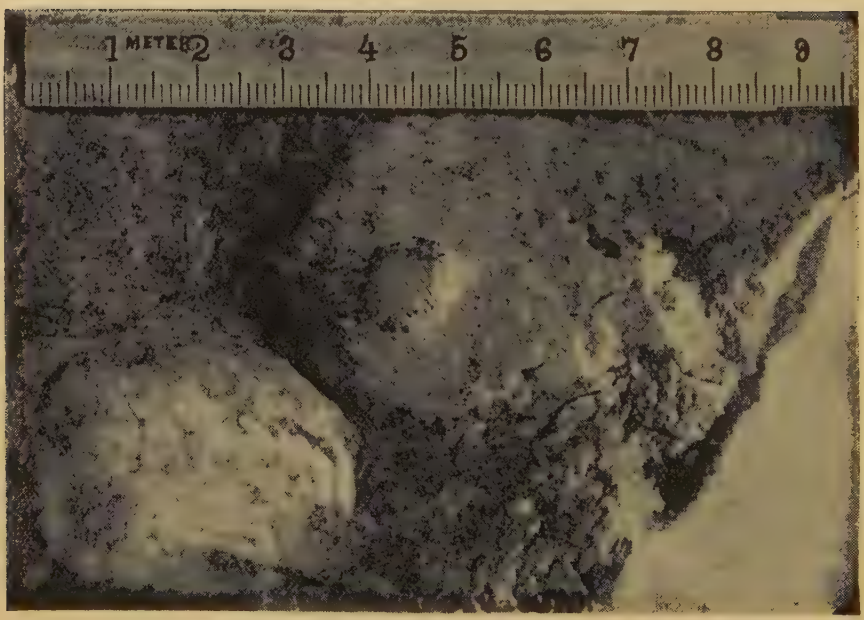

FIG. 2. Early stage of the disease showing two isolated nodules, one protruding from the surface. Both are surrounded by a circumscribed area of emphysema.

In long standing cases the affected parts may be denser and harder to the touch, as a result of fibrotic changes.

No particular changes are seen in other organs; the heart may in some cases be enlarged, sometimes considerably, but on the other hand we have frequently observed hearts of normal size, even in cases where the lung changes are extensive and a heart hypertrophy might have been expected. In long standing cases, when emaciation has reached an advanced stage, gelatinous œdema may be seen on the heart along the *sulcus coronarius*.

The lymph glands of the lungs and mediastinum are not visibly changed, being small and rather dry.

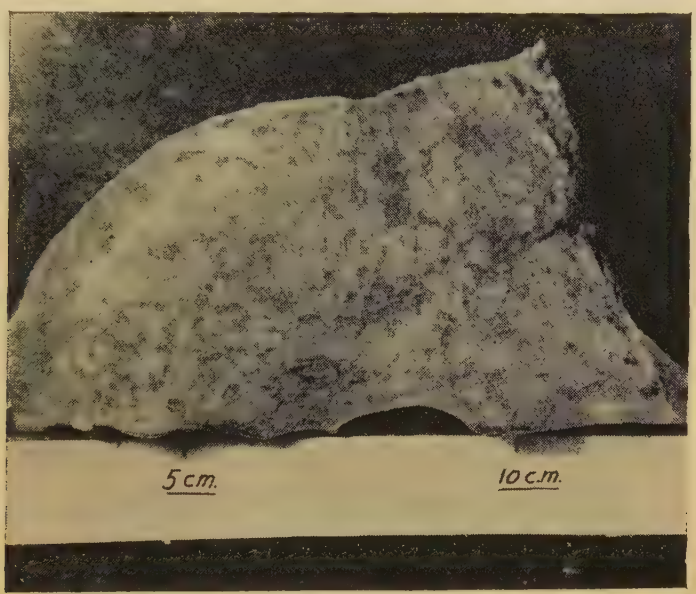

FIG. 3. Early stage of the disease, lung showing numerous small nodules.

No particular lesions are found in the abdominal organs; the liver and gall-bladder, which in contagious pneumonia are constantly enlarged, are of normal size and apparently unaffected in this disease. The spleen is small and there is nothing characteristic about other abdominal organs, all of which appear normal.

The bone marrow exhibits no difference from the normal and there is no apparent anæmia until the last stage of emaciation has been reached.

The brain and spinal chord are apparently unaffected.

Histology.—Stained preparations seen by the naked eye, present numerous patches up to 10 mm. in diameter, which have taken the stain very intensely; most of these are irregular, 2 to 5 mm. in diameter, and stand out in sharp contrast to the intermediary tissue, which is

loose, wide-meshed and fragile, so that the sections are apt to be damaged and torn. Microscopically, the darker coloured patches reveal themselves as islands of adenomatous tissue, composed of papillæ surrounded by more or less gland-like structures. The

FIG. 4. Cut surface of an affected lung showing numerous small nodules.

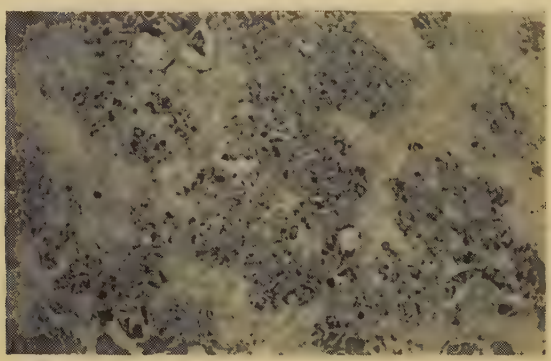

FIG. 5. Affected lung under low magnification showing isolated affected areas in healthy lung tissue.

epithelial cells are regularly arranged and take the form of high cylindrical cells, with a clear, transparent protoplasm, containing an approximately round nucleus. These cells are regularly arranged in a single layer on a narrow band of loose connective tissue. Cut ends

of papillary tufts are frequently seen in the form of isolated islets. The alveoli between the adenomatous islands are sometimes wider than normally, although this is not always so, the walls as a rule being exceptionally thin and frequently ruptured. These alveoli contain a larger or smaller number of mononuclear cells, which are very numerous in some cases, in others very few, so that the spaces between the " adenomas " are almost clear. These cells are very similar, being rather large, containing a small, round nucleus, rather eccentric in position ; occasionally two nuclei may be seen, as though the cell were in the process of division : the protoplasm is frequently fat-laden, containing numerous large granules of fatty substance. Segmented leucocytes are seen in varying amounts : sometimes there are very few, sometimes they are widely dispersed, rarely they occur in groups as in papillary tufts surrounded by epithelial cells, but usually a single one among other cells. The mononuclear cells in the alveoli are seen to stick together in small groups in places ; a structureless, slimy substance, which takes a reddish stain with hæmatoxylin-eosin, exuding from them and apparently gluing them together. These cells appear to arrange themselves along the alveolar walls, forming small projections from the septa. Not infrequently giant-cells are seen, which seem to be formed by nuclear division of the alveolar cells without division of the protoplasm. A conspicuous feature in some instances, where a slight alveolar exudation is seen, is the growth of small epithelial tufts extending from the septa into the alveoli.

Among the epithelial proliferations and especially in the alveoli, erythrocytes are frequently seen in smaller or greater numbers.

In the smaller bronchi papillary tufts of epithelium are frequently seen, sometimes of very considerable extent. In the first stages of the disease the desquamation of bronchial epithelium is very extensive, and the lumen of the bronchus is frequently seen to be full of desquamated epithelium.

The Weigert stain does not reveal any fibrin among the alveolar exudate.

Staining for elastic fibres reveals complete absence of elastic fibres in the adenomatous tissue and a diminution in the alveolar septa.

Bacteriology.—Stained smears from affected tissue as a rule show no bacteria, but sometimes microorganisms are found, which seem to bear no relation to the disease. *Diplococci*, surrounded by a capsule, resembling *Pneumococci*, are among those most frequently met, but are only found in a minority of cases and are not capable of reproducing the disease by inoculation. Briefly stated, many kinds of organisms have been cultivated from the affected lungs, but none with such constancy that causative relationship may be claimed. As a rule, however, no growth is obtained from the lesions when the sheep are killed off, but only from cases reaching the final stage and succumbing to terminal pneumonia, the bacillus of contagious pneumonia, described by Dungal[1] being one of the most frequent invaders.

FIG. 6. Affected lung under low-power magnification showing budding epithelium.

FIG. 7. Affected lung, high-power magnification, showing growth of epithelium supported by loose connective tissue.

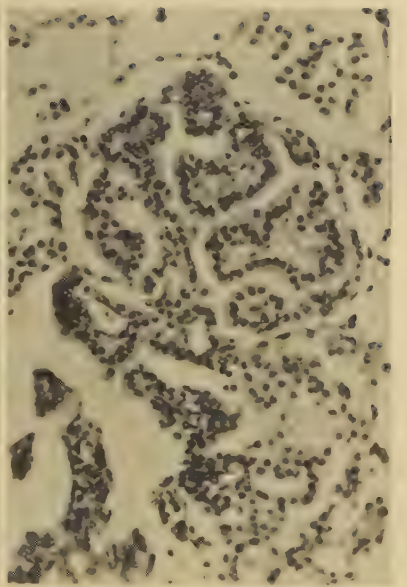

FIG. 8. Papillary outgrowth among alveoli some of which contain the typical desquamated endothelial cells.

Correspondingly, no organisms are found in direct smears from the affected tissue.

Cultivation of Virus.—This was attempted, after the method of Burnett[11] but in spite of repeated attempts with numerous eggs, always with negative results. As this method only allows of incubation for five to six days after inoculation, the time may be too short for lesions to develop on the chorio-allantoic membrane.

Attempts to find Protozoa.—Staining with Giemsa's or Castaneda's stain have revealed nothing which with any probability might be regarded as a protozoan parasite, virus or inclusion body. Dark ground illumination has revealed no spirochætes nor any parasites.

Course of an Outbreak of the Disease in a Flock of Sheep.—When a hitherto healthy stock is affected the course of the disease is strikingly similar in every case. The first signs of infection usually appear during the spring months, April to June, the affected sheep then beginning to show signs of respiratory distress. Being unacquainted with the disease the farmer may suspect nothing and it frequently happens that this first affected sheep is sent along with the others into the mountains, from which it probably never returns. The farmer sees nothing of these sheep until the general collecting in the autumn, and then he notices that the sheep which were housed with the sick one have become ill. From that time onwards his losses slowly and steadily increase. In a flock of 200 to 500 sheep a new case may be discovered daily, and this may go on for weeks and months until 20 to 50 per cent. of the flock are lost.

In January and February, when the sheep are housed on most farms, there is generally a pause, few actually dying during that period, but March rarely passes without increased losses occurring, and in the spring great numbers usually die. All farmers agree that exertion and chill aggravate the disease, rain and storm frequently causing havoc among a sick flock which is turned out to grass, whereas housing may keep the whole flock alive for months.

A survey on 30 farms which had had the disease for about a year showed that 2,017 out of 5,693 sheep, representing a proportion of 35 per cent. had been lost. On all of these farms the disease still progresses, and in another six months the losses are likely to total 50 per cent. of the whole.

Natural Transmission.—The conditions of the great autumn collections appear to be ideal for the transmission of a respiratory disease that is spread by coughing and strenuous breathing, and observations suggest that this mode of spread is of great importance in the propagation of epizootic adenomatosis. In many instances where a case is discovered in a new district, the first sheep affected has been known to have come during the previous autumn from one of the large collecting folds where sick sheep had been observed.

Some examples of transmission from farm to farm are very illuminating and clearly indicative of this mode of transmission as

will be seen from the following case reports, collected from trustworthy farmers :—

Farm Mardarnúpur.—A four-years-old ewe was brought here from another farm, Rútsstadir, on November 2nd, 1936. The disease was noticed a few days after the sheep left the last-mentioned farm and at the end of June the four-years-old ewe at Mardarnúpur showed symptoms of dyspnœa, although the disease did not show in the rest of the flock until the autumn.

On the same date (November 2nd) eight other sheep were driven from Rútsstadir to another farm, Vaglar, and a few days later one of these eight ewes was noticed to be dyspnœic. By the end of the following June 19 out of 60 sheep were lost at this farm.

Whereas in the first instance losses did not begin in the home stock (at Mardarnúpur) until the following autumn, in the second instance, at Vaglar, where the disease appeared in the introduced sheep soon after their arrival, losses began in June. This appears to indicate that the disease is not infectious until symptoms become manifest and that the incubation period is about seven or eight months.

Farm Kornsá.—On the 25.10.36 the farmer introduced a sheep from another farm, Skinnastadir, into his flock, housing it with ten ewes for one or two weeks. He then noticed this sheep to be sick, and slaughtered it ; the lungs on histological examination showing typical adenomatosis. No signs of the disease were, however, observed in members of this farmer's flock until the middle of April, when a ewe, which had been housed with the one from Skinnastadir, was found to be showing typical symptoms. This ewe had not been in contact with any other flock, and an incubation period of some seven months is indicated. Of the nine other ewes which were housed with the introduced sheep, seven were affected with adenomatosis and slaughtered before the end of October, 1937. Here eight out of ten sheep exposed had contracted the disease.

Farm Stokkseyrarsel.—This farm is situated in what was hitherto a clean area in the south, where chances of contact with diseased sheep were small. In October, 1936, a ewe from a distant farm was found to have been driven into the fold belonging to Stokkseyrarsel, and, as is usual in such cases, was sold to the highest bidder, who intended to take it to his farm, some 6 miles from the fold. On account of the dyspnœic condition of the ewe, however, he could not take it all the way to his home, so it was housed in Stokkseyrarsel for two days and nights in a little stall along with four lambs which belonged to the farmer in Stokkseyrarsel. The owner of the ewe then took it home, but after some days, thinking that it would not recover, killed it, when he noticed that the lungs were large and diseased.

The four lambs which were housed with this ewe at Stokkseyrarsel showed no symptoms of disease throughout the following winter and appeared to be healthy when driven into the mountains in June. Two of them did not return, however, in the autumn collection and

their carcasses were afterwards found; the third showed obvious symptoms of disease in September, when it was killed, histological examination showing typical adenomatosis, and shortly afterwards the fourth showed slight symptoms; it was killed and also found to show typical histological lesions.

There was, as far as is known, no possibility of these four lambs coming into contact with the disease anywhere except from contact with the sick ewe which was housed with them, so that the incubation period in this instance must have been eight or nine months, which is unusually long. It must be remembered, however, that the first signs of the disease are so slight that they are easily overlooked, an eventuality which would be particularly probable in a district where there was no known reason for the presence of infection.

Farm Helgavatn.—A lamb came from a diseased farm and was isolated with three others in a small stall. These four sheep did not come into contact with the rest of the flock, which was healthy. At the end of April the lamb which had been in contact with the sick flock showed symptoms of adenomatosis and was killed. Early in April the lambs were mixed with the farm stock for a fortnight, but were at once isolated again as soon as the disease was noticed.

Of the three lambs, which were left, one escaped into the mountains, but the two others were isolated on a small island in the river as soon as they were let out of the stall, being kept there in perfect isolation throughout the whole summer. In the first days of November they were both killed and an adenomatous nodule of walnut size was found in the lung of one, which histologically proved to be adenomatosis. In November six other sheep of the home stock were observed to be affected with adenomatosis.

Besides indicating the long period of incubation this case also suggests that the disease is infectious before symptoms are noticeable, although only a short time before. It will be remembered that the originally infected lamb had been removed as soon as symptoms appeared, and that the lamb just described as showing a very early lesion had only been in contact with the diseased lamb before symptoms appeared.

Farm Hvammur.—A ram from the north, which at that time was a healthy district, was brought to this farm in October, 1935, after having been housed with several sheep on the way; among these were certain sick sheep. This ram was kept in a special house with three other rams and served the ewes in December, but apart from that did not come into contact with them. In March or April this ram showed signs of lung disease, râles being very noticeable and early in June, 1936, it was found dead, no *post-mortem* examination being made.

The rest of the flock showed no sign of disease, and in August of the same year all were apparently healthy. About the middle of September, however, a ram, which had been housed with the ram from the north, showed signs of dyspnœa: on October 4th the

respiratory distress had increased to such a degree that it could hardly walk, and it was killed, the lungs showing typical signs of adenomatosis, which was histologically confirmed. Of the rest of the flock only one lamb was affected (in March, 1937) and a ewe a little later, but in autumn, 1937, wholesale losses began and now this farmer has lost about half of his flock.

The limitation of infection to one particular sheep house is frequently very striking. Where sheep are kept in two or more houses on the same farm, those that have been housed with the first case will always be picked out first by the disease, whereas the remainder of the flock in the other houses may remain in perfect health for a long time after severe losses have been sustained among sheep in the first house. This is all the more remarkable when it is considered that the whole flock may graze the same land together at the same time and even be driven together between farm and pasture. The chance of contracting infection on pasture and outside the house appears to be comparatively slight.

Incubation Period.—In addition to the above we have accumulated a great deal more evidence regarding the period of incubation, which in every instance seems to be several months, and we know of no instance where the disease has appeared shortly after the introduction of a sick sheep. On the following farms the time that elapsed between contact with sick sheep and the appearance of clinical symptoms could be reliably ascertained, as follows :—

Farm.	*Period of Incubation in Months.*	*Farm.*	*Period of Incubation in Months.*
1. Mardarnúpur	7–8	8. Mosfell ...	5½
2. Vaglar ...	7–8	9. Reykir ...	7
3. Kornsá ...	5½	10. Fallandastadir	More than 4½
4. Helgavatn ...	6¼	11. Gilsstadir ...	7
5. Bessastadir ...	8	12. Stokkseyrarsel	9
6. Torfustadir ...	6	13. Höllustadir ...	8
7. Hvammur ...	6		

From this it will be seen that the usual period of incubation is 6 to 8 months.

Hereditary Susceptibility.—All of the farmers agree that certain breeds of sheep are particularly susceptible, a large proportion of their number beginning to show symptoms before they are noticeable in the rest of the flock. There is now the unanimous opinion that the " Gottorp " strain, which is very numerous and widespread is particularly susceptible. On the other hand no strain has been found to be altogether resistant to the disease, although one strain, the " Adalból " strain, which only exists on a few farms, appears to be exceptionally resistant, comparatively few of this breed having been lost. In one outbreak it was observed that a farmer, who had 100 home-bred sheep and 25 of the Adalból strain, lost 80 of the home breed, but only one of the Adalból breed.

A certain farmer who had kept detailed records of the pedigrees of all his sheep was able to supply some interesting data on the losses sustained by the different strains. These data may be summarised as follows :—

Name of Ram.	*Offspring.*		*Deaths per cent.*
	Dead.	*Living.*	
Erpur (Gottorp strain)	77	5	93·9
Ljomi	15	22	40·9
Odinn	40	13	75·5
Kimbi	32	16	66·7
Kolur	24	20	54·5
Adils (Adalból strain)	2	18	10·0
Different	105	69	60·3

The Adalból strain, which is seen to be most resistant of all, is much less prevalent in Iceland than the highly susceptible Gottorp strain.

Comparison with other known Diseases.—The first cases which we investigated showed the presence of a heavy infestation of *Muellerius capillaris*, we observed the adenomatous proliferations in the close vicinity of worms and worm nests, and in some cases saw the larvae of these worms actually in the centre of the epithelial proliferations. The alveolar exudate and the epithelial changes resembled closely the changes described by M'Fadyean[2, 3, 4] and Nieberle who considered them to be caused by parasites. On this account we felt convinced that the Icelandic disease was the same and was caused by *Muellerius capillaris*, which we found with great regularity in the lungs. The only other parasite found in the lungs, was *Dictyocaulus filaria*, but this occurred so irregularly that we considered it could not be responsible for the disease.

The South African Jaagsiekte, described by Mitchell[5] and in later years by de Kock[6, 7] and Cowdry[8, 9], is another disease which is characterised by epithelial proliferations. In clinical course, morbid anatomy and in every respect apart from mortality rate epizootic adenomatosis appears to resemble jaagsiekte : the histological picture is, in fact, so strikingly similar, that if a section of jaagsiekte lung were to be seen in the ordinary course of routine examination of sheeps' lungs in Iceland it would be placed without any hesitation as a naturally occurring case of epizootic adenomatosis.

A third disease, which must also be considered in making comparisons is the Progressive Pneumonia of Montana. This disease was compared with jaagsiekte by Cowdry and Marsh[10] and striking similarities observed. Through the kindness of Prof. Cowdry we have been able to compare sections of lung tissue affected with the Icelandic disease with histological sections of jaagsiekte and Progressive Pneumonia. As already mentioned the jaagsiekte sections show a picture indistinguishable from our cases ; but although the slides of progressive pneumonia show the same kind of exudate cells,

there is a much less pronounced epithelial proliferation in that disease and although we have also met with very similar cases among diseased flocks in Iceland we would consider sections showing the progressive pneumonia picture as "doubtful adenomatosis" if encountered in the course of our routine examination. These "doubtful" cases have, however, never come to our notice among the flocks outside the diseased area.

Investigation of the Association of Parasitic Worms.

The possibility of the association of parasitic lung-worms with the cause of the disease was our first working theory in taking up our investigation of the cause of the disease. The description given in M'Fadyean's papers[2, 3, 4] fitted our observations so closely, and the apparent agreement of de Kock and of Cowdry that the disease which M'Fadyean described was a kind of verminous pneumonia rendered it of importance to go into this matter most carefully. Although, however, our earlier impressions were so strongly in favour of this assumption further observations caused us to suspect that some other agent must be at work.

In the first place *Muellerius capillaris* is of very frequent occurrence in all parts of the country and search showed that heavy infestations could often be found in the complete absence of adenomatosis. In order to ascertain whether the disease might be overlooked in other parts of the country, and at the same time to compare the frequency of *Muellerius* infestation in sheep in various parts, one of us (G. G.) during the autumn of 1936 undertook to examine microscopically 1,000 lungs from a diseased district and another 1,000 from a district where the disease had not yet appeared. This survey revealed an equal degree of *Muellerius* infestation in both areas, namely 91·8 per cent. in the diseased area and 91·4 per cent. in non-diseased districts. The adenomatous lesions were, however, exclusively found in the diseased area.

The findings are set down in the following table :—

Table I.

Comparison of *Muellerius* Infestation in Diseased and in Healthy Sheep, 1936.

1,191 *Lungs from Diseased Flocks.*

	Free from Muellerius.	*Infected with Muellerius.*	
		Lightly.	*Heavily.*
Actual number of lungs examined	208	863	120
Number expressed as a percentage	17·4	72·5	10·1

1,074 *Lungs from Healthy Flocks.*

	Free from Muellerius.	*Infected with Muellerius.*	
		Lightly.	*Heavily.*
Actual number of lungs examined	200	760	114
Number expressed as a percentage	18·6	70·8	10·6

More extensive observations of a similar kind were made with the aid of lay assistance under the supervision of one of us (G. G.) during the autumn of 1937 and are recorded in the following table. The results obtained in the various districts are stated separately and in order that the percentage figures should have some significance only sheep from farms where 50 or more were slaughtered and examined have been included.

In all 51,189 sheep were examined.

TABLE II.

COMPARISON OF *Muellerius* INFECTION IN DISEASED AND IN HEALTHY SHEEP, 1937.

No. of District.	*Per cent. Free from Muellerius.*		*Per cent. infected with Muellerius.*				
			Lightly.		*Heavily.*		
	Healthy Flock.	*Diseased Flock.*	*Healthy Flock.*	*Diseased Flock.*	*Healthy Flock.*	*Diseased Flock.*	
1	3·4	7·0	93·5	91·0	83·0	3·1	Southland Province.
2	9·6	11·6	86·0	70·5	4·5	17·8	
3	11·3	13·8	82·0	70·0	6·7	16·0	
4	8·2	20·5	86·5	75·0	5·2	4·5	
5	5·4	11·0	67·0	77·0	27·6	12·0	
6	9·8	9·4	78·7	81·6	11·5	8·8	
7	5·2	4·3	70·0	73·0	24·8	22·6	
8	9·5	12·5	78·4	97·5	12·0	0	
	3·3	5·9	95·8	93·5	0·8	0·5	Northland Province.
10	0·5	3·0	99·5	95·0	0	1·9	
11	6·3	3·9	90·0	89·0	3·7	7·1	
12	3·9	6·0	91·0	90·7	5·1	3·3	
13	40·0	22·1	48·0	68·0	12·0	9·8	
14	57·5	63·8	42·0	36·2	0·4	0	
15	63·7	47·7	35·5	52·3	0·8	0	
16	6·4	7·1	93·0	92·0	0·8	1·7	
17	16·2	12·2	80·7	81·0	3·0	7·0	
18	6·8	6·0	91·3	91·19	1·8	1·9	Westland Province.
19	6·3	2·9	86·6	91·0	6·9	5·9	
20	10·3	11·5	83·4	79·4	6·2	8·9	
21	10·8	8·8	82·0	87·0	7·2	3·9	
22	8·1	4·6	84·1	80·0	2·6	10·7	
23	0·1	0	66·0	67·5	33·9	32·2	
Average	13·9	8·2	76·9	85·9	9·1	5·9	

Of the total of 51,189 sheep included in this table, 10,081 were from diseased farms and 41,108 from healthy farms.

It was difficult to fit in these findings with the assumption that worms were the only cause of the disease, but as the opinion of that most experienced pathologist M'Fadyean, as gathered from his three papers[1, 2, 3] appeared to be so strongly in favour of the cause of the disease by parasitic worms, some further observations were carried out by one of us (E. L. T.).

The first part of this enquiry aimed at eliminating the possibility of a new and more pathogenic species of lungworm being concerned. For this purpose the tails of a number of male worms were teased out of the affected tissue of several lungs but in every case the worms were found to be referable to the species *Muellerius capillaris*, the best known member of this group of parasitic lung worms, and the one which is most prevalent in the British Isles.

Observations were next made on the relative concentration of lungworms in diseased and healthy parts of affected lungs and for this purpose pieces of approximately 10 grammes in weight were taken from healthy portions of the lung, others from diseased portions and the contained *Muellerius* larvæ collected by mincing, shaking with glass beads, washing and screening. The larvæ from the various lots were then counted, but no indication was obtained of the occurrence of a heavier infestation in diseased parts than in healthy parts of the lung. The observation of one of us (N. D.) of the different distribution in the lungs of the earlier lesions of adenomatosis and the lesions of *Muellerius* infestation agrees with this conclusion, the adenomatosis usually developing along the ventral margin, and the worm nodules in the dorsal border of the lungs.

A third possibility to be investigated was that of the occurrence of a mass invasion of fourth-stage lungworm larvæ as a cause of the disease. A search was, therefore, made for the young worms, portions of affected lung being minced and larvæ separated by a method similar to that above mentioned, but though a considerable amount of material was examined and many thousands of first stage larvæ were separated from the tissues no larvæ belonging to any other stage was recovered.

Subsequent observations in the field strongly suggest that infection of Icelandic sheep with these worms can only take place during the summer and autumn, as a most careful search revealed the presence of the eggs of land molluscs only. These were found to be very numerous indeed, deeply situated in the herbage and often buried just beneath the surface of the soil. Only one adult was found and this occurred underneath a stone which was lying on a bank of earth covering a hot water pipe conveying hot water to the farm from a nearly hot spring.

The possibility of the transmission of the disease by lungworms was investigated by bringing about an experimental infestation with *Muellerius* of Icelandic origin in two lambs kept in isolation at the British Ministry of Agriculture's laboratories at Weybridge. Affected lungs were dispatched to England, packed in ice. When the lungs were thawed the larvæ were found to be active and apparently normal and were collected in as large a number as possible and were fed to worm-free snails between February 24th and March 25th. On April 21st, 24th and 30th these snails, which were found on dissection to have become infected, were fed to the two lambs. No. 949 received eight infected snails in all, and No. 950 received 13. The fæces of

the lambs were then examined weekly for evidence of infection, but no larvæ were recovered until July 28th. Lamb No. 950 was slaughtered on July 29th and on *post-mortem* examination large numbers of small, hard, caseous, and calcarious nodules were found throughout the lung, indicating the abortive development of numerous worms ; there was, however, no sign of adenomatosis.

The other lamb was kept in isolation indoors and fed only on dry food until December 8th when it was slaughtered. During the whole of that time it continued to pass the larvæ of *Muellerius* in the fæces, and several centres of infection containing living worms were found in the lungs on *post-mortem* examination ; but there was no sign of adenomatosis.

Further observations were subsequently made among flocks on farms where the disease occurred, estimations being made on the numbers of *Muellerius* larvæ per gramme of fæces from about a dozen sheep on each farm. The results of the examination are set down in the following table :—

TABLE III.

SHOWING AVERAGE NUMBERS OF *Muellerius* LARVÆ PER GRAMME OF FÆCES AMONG GROUPS OF SHEEP FROM HEALTHY AND AFFECTED FLOCKS.

No. of Farm.	*Proportional Mortality from Adenomatosis.*	*Number of Muellerius Larvæ per Gramme of Fæces.*	
		Diseased Flocks.	*Healthy Flocks.*
1	?	200	
2	?	100	
3	?	300	
4	100/260	500	
5	150/250	253	
6	38/80	33	
7	100/200	33	
8	40/130	33	
9	150/300	500	
10	10/280	*200	
11			266
12			350
13			200
14			133
15			233
16			50
17			200
18			400
19			50

* 700 larvae per gramme of fæces were found in one sheep, slaughtered here at the time of our visit and found to be free from adenomatosis but heavily infected with lungworms.

At the conclusion of these observations it was regarded by us as very improbable that parasitic lungworms had any important connection with the causation or development of this disease or with the rate of mortality resulting from it.

Search for Evidence of the Association of a Microscopic or Ultra Microscopic Agent.

Transmission Experiments.—Eight sheep from a healthy area were on November 26th, 1936, put into a house with 20 sick sheep, which were being used for observational purposes. One of these (No. 432) received an intratracheal injection of 1 c.c. of a saline emulsion of affected lung on November 30th. During the whole of the winter these sheep were stall fed and were not allowed to go out on to the pasture. The original number of sick sheep was maintained by placing others into the stalls as deaths occurred, but at no time throughout the whole of the winter did any healthy sheep show signs of having contracted the disease. In May, one of the test sheep was observed to have lost considerable weight, but definite signs of adenomatosis could not be detected when the sheep were turned out on to pasture on June 20th. In the beginning of July this same sheep showed accelerated breathing and slight dyspnœa, and in August the symptoms of adenomatosis were quite definite. This sheep was killed on August 30th, the autopsy revealing extensive adenomatous nodules in both lungs, these nodules proving to be histologically typical.

The incubation period in this case is thus seen to have been about seven months. Of the other sheep six became affected, the symptoms appearing in August, and the beginning of September, and on *post-mortem* examination all were found to exhibit a nodule of about the size of a hen's egg in one lung.

One of these sheep was a yearling, which from birth had been protected from worm infestation by stall-feeding on milk and on autoclaved grass, never having been allowed out to pasture until it was a year old. On *post-mortem* examination this sheep was found to have a very slight *Muellerius* infestation (acquired between June 20th and September 20th), and on *post-mortem* examination an adenomatous nodule of about the size of a duck's egg was found in one lung accompanied by smaller nodules.

It is a curious observation that some lambs from a farm where the disease had been causing great loss for more than twelve months (a farm adjoining Deildartunga) and which were housed in the same stall throughout the winter, did not contract infection. They were killed at the end of September, after having been in close contact with the sick sheep for ten months, but no trace of adenomatosis was found.

Whether this resistance to the disease may be attributed to acquired immunity on the farm is not certain, but the explanation, need not necessarily concern susceptibility and acquired immunity as these lambs were the offspring of sheep that had survived the epidemic and might therefore have inherited the natural resistance of their parents.

Transmission by Inoculate into the Lung.

On March 6th, 1937, 5 grammes of typical adenomatous tissue was ground with sterile sand in a mortar, 10 c.c. of saline being added and the emulsion sieved through gauze to remove the coarser particles. This filtrate was injected intrapulmonally into three lambs, 2 c.c. into each. The lambs appeared perfectly healthy after the injection, showing neither fever nor accelerated breathing, and they remained apparently healthy until October when one of them showed suspicious symptoms, slight râles being heard in the right lung. This lamb was killed on October 22nd, the autopsy revealing an extensive lesion in the right posterior lobe and smaller patches in the right middle lobe and in the apical lobe. In the left lobe a nodule of the size of a hen's egg was seen running along the bifurcation of the anterior bronchus.

The other two lambs were killed on the following day, but no sign of adenomatosis was found in them.

Attempted Transmission by a Filtrate of Triturated Lung.

On March 11th, 1937, a portion of adenomatous lung tissue, weighing 12 grammes, was ground with sterile sand in a mortar, diluted with 50 c.c. saline, sieved through gauze and subsequently filtered through a Seitz filter. Of this filtrate 2 c.c. were subsequently injected intrapulmonally into five healthy sheep. On October 22nd and 23rd all of these sheep were killed but no lesions were found.

These experiments showed that the disease may be transmitted by intrapulmonary injection, for there could hardly be any doubt that the one of the three sheep was infected by the injection, as the lesion was seen to spread from the site of the puncture.

The negative result from the filtrate cannot, however, be regarded as excluding the possibility of a causation by a filterable virus, as it is obvious that infection is not easily brought about by intrapulmonary inoculation, only one out of three sheep having become infected as a result of the inoculation of unfiltered material.

The possibility of Infection being Conveyed by Hay.

In order to investigate this important practical point hay was obtained from pastures where sick sheep had been grazing on the badly infected farms.

A healthy flock of 40 sheep was divided in two, 20 receiving this hay untreated and the other half the same hay heated to 100° C. for 30 minutes. The sheep were kept on this hay and stall fed throughout the winter, at the end of which period the group on heated hay was found to be in a definitely poorer condition than the other group. One sheep in this group fell sick in June while being stall fed, it could hardly stand and was killed after two days in this condition, but on *post-mortem* examination no signs of adenomatosis could be found nor did any symptoms of the disease appear in any of the other sheep. A *post-mortem* examination of a second sheep also showed negative results.

Bacteria Isolated from Adenomatous lesions.

Several kinds of bacteria have been isolated from the lung lesion, but none with sufficient frequency to be reasonably supposed to be the cause of the disease.

We have repeatedly found a Gram-negative diplococcus, provided with a capsule, resembling the organism of contagious pneumonia. One of these strains, which we found in pure culture in a typical case was injected intrapulmonally into a ewe and its lamb, but after eight months both were found to be perfectly healthy. Many similar strains were tested on mice (which are very susceptible to the organism of contagious pneumonia) and one strain was found which killed mice regularly after three to four weeks. This organism, however, produced no visible lung changes in the mice and proved to be non-pathogenic to sheep.

Most of the micro-organisms which we have isolated from the lung lesions have proved non-pathogenic. A streptococcus, which we isolated from a typical case, was injected intrapulmonally into three sheep and intratracheally into two others, but without any result.

Pathogenicity for other Animals.—The disease, has, so far, not occurred in any farm animal other than the sheep. Adenomatous tissue has been injected intraperitoneally, intrapulmonally and intravenously into white mice and rabbits, but without any result.

Discussion.—The disease here described corresponds closely to two previously described diseases ,*viz.*, the peculiar type of verminous pneumonia of M'Fadyean and jaagsiekte, as described by de Kock, Cowdry and others. It also bears considerable resemblance to the progressive pneumonia, described by Cowdry. The question naturally arises whether all these diseases, described under various names, are one and the same disease, having one common cause.

The suggestion of de Kock, that jaagsiekte may be caused by different microorganisms, which all produce the same or similar lesions, namely epithelial proliferations in the lungs, cannot be valid in our cases from the following reasons : (1) The epidemic nature of the disease which can be traced to one centre in Iceland to which it appears to have been imported. (2) The disease has not been observed in parts of the country where there has been no communication with sick sheep, although contagious pneumonia and verminous pneumonia occur there. (3) It is very unlikely that various microorganisms should heve been imported at the same time and cause this disease by spreading simultaneously, and it is known from previous observation that other bacterial pneumonias, which are well known in Iceland, do not produce similar changes. (4) In the vast majority of affected sheep which are killed before the terminal pneumonia occurs, no microorganisms are found.

The most reasonable hypothesis seems to be that some new microorganisms or ultra-visible virus has been imported, causing these very

characteristic histological changes and that there is only one etiological factor for this disease.

The histological pictures of our cases are so similar to jaagsiekte and the " verminous pneumonia," described by M'Fadyean, that we are unable to see any difference which might serve to distinguish the one from the other either microscopically or macroscopically. Cowdry states after comparing sections of verminous pneumonia sent to him by M'Fadyean with jaagsiekte that " it would be difficult, through microscopic examination alone, to distinguish the lesions of this disease from those observations here recorded."

The presence of worms in the lungs, particularly when seen in great numbers, is very suggestive of their causative relationship to the lesions in question, but their presence is no proof of any part played in causing the lesions. On the contrary, the observations recorded in this paper appear to disprove that point.

The great similarity between jaagsiekte, verminous pneumonia and Icelandic adenomatosis therefore leads us to suppose that all three are in reality one and the same disease.

The higher rate of mortality among Icelandic sheep may be the result of a peculiar susceptibility of the sheep there, but it is not improbable that the special methods of sheep management, and particularly the folding together of large numbers in the autumn and the close housing during the winter also play an important part.

Summary.

An evidently infectious disease which seems to have been newly introduced into Iceland is causing very heavy losses among sheep. During the course of about one and a half years it usually causes the loss of about 50 per cent. of an affected flock. This disease runs a very chronic course, and has an incubation period of six to eight months, or even more. The chief clinical symptoms are respiratory distress, bronchial râles, nasal flow of mucous, frothy, watery exudate in the bronchi, but no fever. The histological picture is very distinct, consisting of epithelial proliferations forming papillary adenomatous tissue, and a more or less pronounced exudation of mononuclear cells into the alveoli.

The histological picture corresponds very closely to that of jaagsiekte and M'Fadyean's " verminous pneumonia," also in a certain degree to Cowdry's progressive pneumonia of sheep.

Transmission has been easily affected by housing sheep together, but intrapulmonary inoculation was only successful in transmitting the disease once out of three attempts made.

Up to the present the disease has not been transmitted by the injection of bacteria-free filtrates, but no microorganism has been found which can reasonably be regarded as the causative agent. The possibility of its cause being due to the presence of parasitic lung-worms appears to have been eliminated.

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ANATOMICAL AND EXPERIMENTAL STUDY OF THE TEAT OF THE COW WITH PARTICULAR REFERENCE TO STREPTOCOCCAL MASTITIS

By Thomas Johnston, M.R.C.V.S., D.V.S.M.

The following preliminary observations on the teat of the cow were made to determine if any information could be obtained from this line of investigation which might throw some light on the conditions governing the entrance of streptococci into, and their subsequent behaviour in, the milk sinus.

Although it is now generally agreed that under natural conditions pathogenic streptococci reach the udder tissue by way of the teat duct, attempts to establish infection experimentally by the application of infective material to the end of the teat have frequently proved unsuccessful[1, 2, 3]. By exposing normal teats to infective material Christiansen and Nielson[3] were unable to produce mastitis, but could do so readily when the closing mechanism was impaired by some alteration in the structure of the duct resulting from injury either accidental or produced experimentally. This indicates that infection of the udder is in some measure controlled by the closing mechanism of the teat. Christiansen and Nielson conclude, however, that under natural conditions there must be some other factors, at present unknown, governing the entrance of bacteria through the teat canal, as most cases of streptococcal mastitis develop, when there is no obvious impairment of the sphincter.

The reaction of the udder to pathogenic streptococci introduced experimentally into the teat sinus has been studied by a number of investigators[4, 5, 6, 7, 8] and the results indicate that infection by this means is not difficult. In most cases, however, the gland has been exposed to more severe infection than is likely to obtain under natural conditions. When the infected material was highly diluted, Jones and Little[9] found that, in most cases, several inoculations were necessary before typical mastitis developed and they suggested that, under natural conditions, the udder must first be sensitised by frequent exposure to infection before organisms can establish themselves. This aspect of the subject will be discussed later.

According to Zieger[10] some strains of cows are more resistant than others and he is of the opinion that the resistance is hereditary. If such a resistance exists, the question arises whether it is immunological or depends upon some transmissible character in the structure of the udder or teats.

The first part of this study deals with the anatomy of the teat, and features, which appear to have some bearing on the pathogenesis of mastitis, are emphasised. Later, experiments are recorded indicating the manner in which some of the structural features of the teat may predispose the udder to infection. These observations are

based on the examination of 324 teats obtained at random from all types and breeds of cattle.

Part I.—Anatomical.

The teat of the cow consists largely of bundles of unstriped muscle tissue and blood vessels arranged more or less longitudinally and held loosely together by fibrous tissue (Photo 1). Near the free extremity the muscle bundles are arranged in a circular manner around the duct to form a sphincter (Photo 2, A and Fig. 1).

The lumen of the teat is divisible into two parts (Fig. 1).

1. The teat sinus, which is really a downward extension of the lactiferous sinus.

2. The duct, which drains the sinus and opens at the free end of the teat.

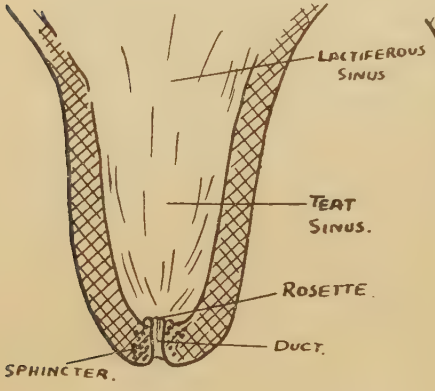

Fig. 1.

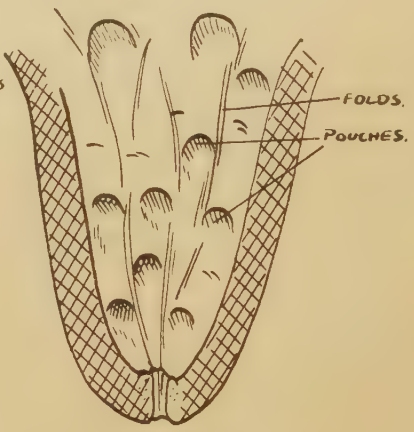

Fig. 2.

Teat Sinus.

The lining membrane of the sinus is a thin and delicate structure with an epithelium consisting of one or two layers of cells. The superficial cells are columnar or cubical and the deep cells flattened (Photo 3). The underlying connective tissue is loose and contains a variable amount of elastic tissue.

In some teats the lining membrane of the sinus shows a number of pouches which probably represent aborted glandular offshoots. These are about $\frac{1}{8}$ in. deep but occasionally one encounters pouches $\frac{1}{4}$ in. deep to $\frac{1}{2}$ in. deep, extending in an upward direction from the opening (Fig. 2). The lining membrane forms a thin cusp at the opening of the pouches. Owing to the paleness of the membrane and the thinness of the cusps it is often difficult to detect the presence of the pouches.

In one teat the sinus was divided longitudinally by a complete membrane into two compartments of unequal size, the smaller opening into the main duct about the junction of the middle and

distal thirds (Fig. 3). The sinus in another case was divided equally by a complete membrane, each compartment opening into the upper end of the duct (Fig. 4). Fig. 5 shows another variation of the pouches described above, *viz.*, an offshoot about ¼ in. long arising from the duct.

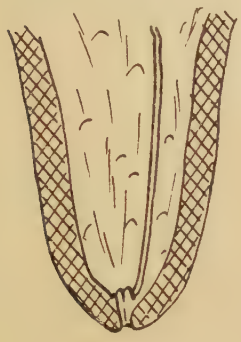

Fig. 3.

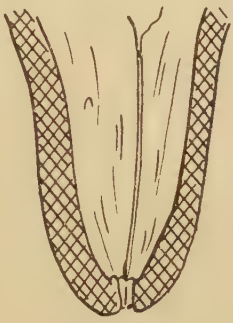

Fig. 4.

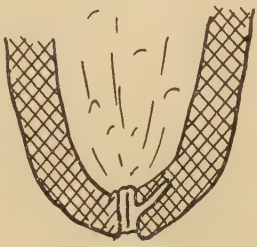

Fig. 5.

Another feature of the lining membrane of the sinus is the presence of folds. Variable in number and size, they may be confined to the lower part or extend the whole length of the sinus. They do not appear to be merely irregular folds of slack membrane, as it is difficult to efface them entirely by stretching the membrane. Sometimes they divide and join other folds as they course longitudinally towards the upper part of the sinus, where some of them encircle the openings of the lactiferous ducts (Fig. 2). In a few cases there are present rows of papillæ, the distribution of which seems to indicate that they bear some relation to the folds (Fig. 6). Two teats had in the upper part of the sinus a ring of stratified squamous epithelium ¼ in. wide, entirely different from the surrounding epithelium but similar to that of the duct; it was noted that the course of several folds, which were also present, was interrupted but not altered by this ring (Fig. 7, A).

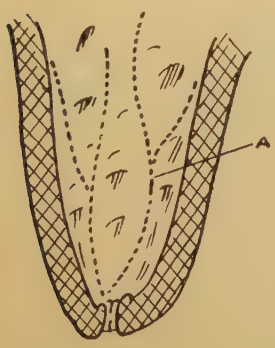

Fig. 6.

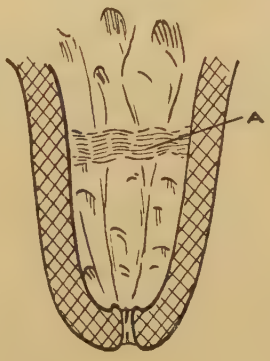

Fig. 7.

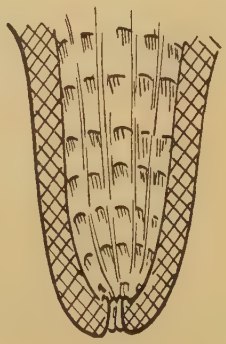

Fig. 8.

An interesting arrangement of folds and pouches was observed in four teats. Four prominent folds radiated from the upper end of the teat duct and divided the wall of the sinus into four equal areas, each of which presented pouches arranged regularly one above the other as shown in Fig. 8. This formation of folds and pouches might suggest an original tendency of the teat to form four distinct cavities.

In many teats the lining membrane of the sinus is smooth and entirely devoid of folds and pouches.

Teat Duct.

The lining membrane of the duct has a stratified squamous epithelium which is variable in thickness (Photo 2, c). At the external opening the epithelium merges imperceptibly into that of the skin, although microscopically a fringe of epithelial processes may frequently be observed at this point. The epithelium terminates abruptly at the internal opening of the duct where it gives place to the thin epithelium of the sinus. At this point the duct epithelium in most teats presents a number of variable projections forming what has been described as a " rosette " (Fig. 1).

Length of Duct.

The average length of the ducts examined was 8·6 mm. The longest duct was 13 mm. and the shortest 5 mm.

No definite relation could be found between the length of the teat and its duct.

Circumference of the Duct.

The circumference is variable at different points in the length of the duct. The greatest circumference is usually at the upper end, while the smallest is usually at some point in the lower half of the duct. Measurements were made after slitting open the duct and the following diagrams and numbers give some idea of the various types of ducts and the frequency with which they were encountered. This classification is only approximate (Fig. 9).

Fig. 9.

It will be seen that only in a small number of teats was the circumference uniform throughout the length of the duct.

The circumference of the duct at its narrowest point is the only one likely to be of any significance in controlling the passage of organisms into the teat and is the one referred to in the following remarks. The widest duct had a minimum circumference of 11 mm. and the narrowest 4 mm. The average minimum circumference was 7·2 mm.

No relation could be found between the length of the duct and its minimum circumference. For example, one duct 10 mm. long had a circumference of 5 mm. while other ducts 6 and 9 mm. long had circumferences of 8 mm. and 10 mm. respectively.

The Skin of the Teat.

The skin of the teat has an extremely thin epithelium—in some parts only two cells deep—with a thin and fragile stratum corneum As the udder of the cow is now larger and more exposed to injury as a result of selective breeding and intensive milk production, the thinness of the skin becomes of some practical importance.

Part II.—Experimental.

That pouches and other irregularities in the lining membrane of the teat sinus may act as reservoirs for organisms is indicated by the following experiments :—

Experiment 1.—The apparatus employed was a five gill bottle with a hole in the bottom; a cow's teat with pouches; powdered carbon. The teat was filled with carbon, manipulated to ensure that carbon reached the pouches, and then fixed to the neck of the inverted bottle (Fig. 10). Water was poured into the bottle and " milked " through the teat. When about a gallon of water had been passed through the teat, the latter was removed, opened and the sinus examined. It was found that a considerable quantity of carbon had been retained in the pouches and there was practically none elsewhere except for a few grains round the rosette.

Experiment 2.—The above experiment was repeated with a teat devoid of pouches. The carbon was completely flushed away except for a few grains adhering to the rosette.

These experiments were carried out several times with the same results. It is interesting to note that in one teat the carbon adhered to an abrasion about ¼ in. in diameter, although there was no trace of it elsewhere.

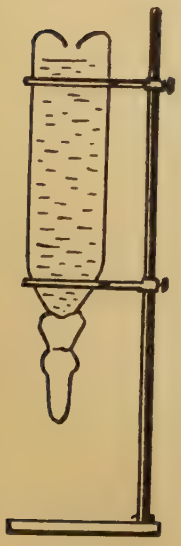

Fig. 10.

The above experiments were designed, as far as possible, to represent the conditions to which organisms in the teat are subjected during the milking process. Although it is obvious that this attempt to reproduce natural conditions is imperfect in several details, there is no reason to suppose that the forces to which the carbon particles are subjected in the experiments are essentially different from those acting on bacteria in the teat when a cow is milked.

It was observed during the " milking " process that when the upper end of the teat was not effectively sealed before applying pressure, a stream of carbon passed up into the bottle. In this way it would appear possible for bacteria in the teat to be driven up mechanically into the lactiferous sinus and ducts which drain into it.

If pressure is applied to the teat in such a way that the seal is broken, one would expect that the sudden release of this pressure before the sinus is emptied will create a temporary vacuum with consequent backflow of milk from the duct into the sinus. The following experiment supports this view :—

Experiment 3.—Same apparatus as before. A teat was fixed to the neck of the bottle which was filled with water. Sufficient pressure was applied to the teat to cause a few drops of water to escape and at the same time some carbon was applied to the end of the teat. The pressure was then suddenly released. This was repeated about 20 times and the teat was then removed, opened and examined. This experiment was carried out with 10 different teats and in four carbon was detected in the sinus, around the rosette and in the duct. In two other cases grains of carbon were found in the duct only.

When the cow moves or walks, especially if the udder is hefted, the teat is subjected to conditions which are similar to those reproduced in the above experiments, and it therefore appears that organisms at the end of the teat may in this way reach the sinus.

Discussion.

Little is known of the conditions which govern the entrance of pathogenic streptococci into the udder and their subsequent behaviour prior to the development of mastitis.

A number of workers have shown that the application of infective material to the end of the teat is not in itself sufficient to establish infection. Christiansen and Neilson showed that impairment of the closing mechanism by injury facilitated the entrance of organisms into the sinus but, as natural infection occurs when the teat is normal, concluded that some other factors, at present obscure, are at work. The opening of a normal duct under excessive pressure when, for example, the udder is hefted or the teat is manipulated during the milking process, may be considered an impairment of the closing mechanism, even if it is temporary and physiological, and Experiment 3 indicates that this at least is one of the factors on which infection depends. Although the apparatus used is hardly an exact reproduction of the udder, the forces which, in this experiment[3], carried the carbon particles into the teat sinus, are also likely to operate under natural conditions. When the udder is overstocked and the teat sphincter is subjected to considerable strain, a column of milk will be constantly flowing up and down the duct with additional and intermittent pressure from the legs when the cow moves, and, if the end of the teat is contaminated with bacteria, they will be drawn up into the sinus.

Although facts have been given regarding the length, width, and shape of the teat duct, their significance in relation to mastitis is not clear. While one would expect organisms to pass more readily into the sinus when the duct is short and wide, it is obvious that the

efficiency of the duct as a seal cannot be accurately measured in terms of millimetres, as the strength and tone of the sphincter must be considered. In fact, if organisms are carried mechanically into the sinus by the back flow of milk from the duct when the latter opens and closes under varying pressure, it would appear that the strength of the sphincter is of more importance than the dimensions of the duct. If, for some reason (general illness, tuberculosis or other debilitating disease), the sphincter is weakened, less pressure would be necessary to open the duct and organisms would therefore have more opportunity of reaching the sinus. Some alteration or deformity of the duct which prevents the sphincter, however powerful, from closing the lumen would have the same effect.

Although pathogenic streptococci may be recovered with equal frequency from the anterior and posterior quarters, experience shows that the degree of infection is greater in the hindquarters (Table I). This may be explained by consideration of the position of the anterior

TABLE I.

STREPTOCOCCAL MASTITIS—QUARTERS CLINICALLY AFFECTED.
(168 cases examined.)

Quarters affected.	*R.P.*	*L.P.*	*R.A.*	*L.A.*
Right posterior quarter ...	108			
Associated with mastitis in		36	4	
Left posterior quarter ...		80		
Associated with mastitis in	36			
Right anterior quarter ...			12	
Associated with mastitis in	4			4
Left anterior quarter ...				12
Associated with mastitis in			4	

and posterior quarters in relation to the hind legs. The posterior quarters are more likely to be subjected to excessive leg pressure than the fore quarters when the cow walks or lies down or even moves about in her stall, especially if the udder is large and hefted. Consequently, the sphincter or closing mechanism of the posterior teats will be impaired more frequently than that of the anterior teats, and the opportunity for the passage of organisms into the posterior quarters will therefore be correspondingly increased.

The experimental findings of Jones and Little[9] show that, when infective material reaches the teat sinus, cows differ markedly in their susceptibility, and this applies even to individual quarters of the same cow. This supports the view that the difficulty of establishing infection when infective material is applied to the end of normal teats is not entirely due to the efficiency of the closing mechanism and that other factors may operate after the organisms have reached the sinus. Jones and Little suggest that sensitisation of the udder by invading organisms is a necessary forerunner of mastitis but, if the teats had

been examined, it seems possible that their experimental findings might have been explained by the existence of some peculiarity in the structure of the lining membrane (Experiments 1 and 2).

So long as the cow is milking normally, the flow of milk from an infected quarter will tend to check mastitis by the removal of invading organisms. This disinfection of the udder and teats during the milking process will be more or less efficient according to the opportunities afforded for the lodgment of organisms, and this in turn depends on the structure of the lining membrane of the sinus. Experiment 1, for example, indicates that the pouches on the lining membrane will act as reservoirs where organisms may remain untouched by the flow of milk. In Experiment 2 failure to dislodge carbon particles from an abrasion $\frac{1}{4}$ in. in diameter on the lining membrane of the teat suggests that any such roughness may also act as a reservoir for invading organisms; injury of the delicate lining membrane may therefore predispose the udder to mastitis.

It was found when carrying out Experiments 1 and 2 that if, during the " milking " process, sufficient pressure was not applied to the upper end of the teat a stream of carbon passed into the container. Under natural conditions organisms may thus be driven up into the udder. It therefore seems possible that this is another factor in the causation of mastitis and that mastitis in some cases may be prevented or its effects reduced if this back flow is avoided, especially when drawing off the foremilk.

The thinness of the skin was noted in Part I of this study. It is not considered that infection reaches the udder through cutaneous wounds or sores, but these may harbour pathogenic streptococci and, if they occur, especially near the end of the teat, the risk of infection *via* the teat duct is increased.

The foregoing observations and experiments indicate that the teat with a normal duct of average dimensions, strong sphincter and devoid of pouches and other irregularities, will be a more effective barrier to infection than one with a very short and easily dilated duct and whose sinus presents many pouches, etc. These are anatomical features which may be transmissible. If this is so it should be possible by selective breeding to increase to some extent the resistance against streptococcal mastitis. Zieger suggests that some strains of cattle possess genetic factors of resistance; this resistance may be of a specific immunological nature, but the possibility that it may be anatomical should be considered in further investigations.

Summary and Conclusions.

Some anatomical features of the teat are noted and discussed.

An attempt has been made to determine experimentally the probable behaviour of streptococci leading up to an attack of mastitis.

The findings suggest that mastitis depends on a combination of factors some of which are associated with the anatomy of the teat and certain conditions to which the udder and teats are subjected.

A thorough and complete study of the anatomy and physiology of the udder and teats as a basis of further research work on mastitis is indicated.

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Note.

A recent article on Mastitis by Little[11] has just come to my notice and is of considerable interest. It deals with two aspects of mastitis, *viz.*: —

(1) The Significance of the Dose Factor in the production of experimental mastitis.

(2) The production of mastitis by the suction of streptococci into the duct of the teat.

First calf heifers were inoculated with typical mastitis streptococci. When pure culture or 10^{-1} dilution was employed 12 quarters of five heifers developed masititis after a single inoculation. When fewer organisms were inoculated it became more difficult to establish infection, more inoculations being required.

[The development of mastitis after a single dose of pure culture indicates that an allergic sensitisation of the lining membrane of the sinus (Jones and Little) does not necessarily precede mastitis. It is possible, however, that a smaller dose may set up a mild catarrh with desquamation of the lining cells and consequent roughening of the surface. Although this may pass off unnoticed, the quarter, in view of the altered state of the lining membrane, may thus be rendered more susceptible to mastitis. T. J.]

It is also shown that when the teat is inserted into a rubber bag containing a culture of streptococci the entrance of organisms into the sinus is facilitated by manipulation of the teat. From this it appears that organisms may be carried into the teat sinus by suction. In this way mastitis was produced in two six year old cows and one heifer. Little found that the heifer was more resistant than the cows and states that the resistance of the heifer is probably related to the intact duct and sphincter. (Other probable contributing factors, *viz.*, nature of the lining membrane, presence or absence of pathological change, and certain physiological conditions have been discussed by T.J.)

JAAGSIEKTE

By Sir John M'Fadyean.

Wm. Robertson, Bacteriologist to the Department of Agriculture, Cape Town, appears to have been the first to make a scientific study of this disease, *Jour. Comp. Path. & Ther.*, **17**, 1904.

He described the lesions as a peculiar form of pneumonia, in which the hardening and consolidation of the lungs was not preceded by the usual stages of congestion, hepatisation, etc., and there was an almost entire absence of fever. The course of the disease was described as follows :—

The first symptom usually noticed is a cough, with marked shortness in depth and increase in number of respirations. These symptoms increase in severity at the disease progresses, until the least movement causes the animal to pant for breath, heave at the flanks and lie down completely exhausted. The animal looks as if it had been over-driven, and that is the impression which the casual observer might get if he saw two or three affected sheep trailing after the flock. The course of the disease is more rapid in winter than in summer, and careful treatment and nursing prolong the animal's life.

The Chief Veterinary Surgeon shared the opinion that the disease was of a contagious nature and recommended the slaughter of all affected animals.

The author found that the lungs and their lymphatic glands were alone affected; the latter were enlarged, pale and œdematous. The lungs show more or less extensive areas of consolidation; sometimes there seems to be only one centre or area of infection, but in other cases the infection appears to have started from many different centres. The affected part is quite solid and of a darker colour than normal lung; in some cases it cuts like bacon, and in advanced cases like cartilage. The bronchi persist but the lumen is often occluded. The consolidated parts do not appear either congested or swollen.

In typical cases of the disease there does not appear to be any tendency towards breaking down of the lung tissue or formation of abscesses. The pleura is not affected in the early stages. but later it may become thickened at some places and adhesions may be formed between the lung and the chest wall.

The affected parts of the lung showed no tendency to undergo degeneration, caseation, or the formation of abscesses.

Robertson's article is accompanied by two microphotographs which are of considerable value as showing the real nature of the lesions. They are not in accord with the view that the new tissue is of the nature of an adenoma.

Robertson described the alterations as those of a catarrhal pneumonia, in which the alveoli were plugged with exudate " almost entirely composed of proliferated cells from the alveolar lining," mixed with a few leucocytes. The affection appeared to spread steadily from the diseased to the healthy lung.

He found in smears from the affected lungs peculiar bodies which he was unable to find in healthy lungs or in the healthy parts of lungs affected with the disease. He did not claim that these bodies were the causal parasites of the disease but he suspected them to belong to the sporozoa.

He had "completely failed to convey the disease from sick to healthy animals by cohabitation, feeding, and all varieties of inoculation methods, both with blood and affected tissues."

Dr. E. V. Cowdry made a comprehensive study of jaagsiekte, first in the Transvaal, where there was abundant opportunity to observe the course of the disease at first hand during life and *post-mortem*, and secondly at the Laboratories of the Rockefeller Institute, New York, where the collected material could be subjected to microscopic study. The results were published in two Reports (1925), the first of which deals with the Primary Lesions and the second with the Origin of the Epithelial Proliferations and the Subsequent Changes (*Jour. of Exp. Med.*, **42,** 1925). In the present connection it is necessary to refer only to his views regarding the histology of the typical lung lesions in jaagsiekte.

In Dr. Cowdry's opinion the primary lesions develop through a local congestion of the alveolar capillaries accompanied by the accumulation of macrophages and of lymphocytes in the inter-alveolar tissue and the penetration of the former in large numbers into the alveolar lumina. It is only in portions of the lungs modified in this way that the "epithelial proliferations which are so characteristic of jaagsiekte arise." The proliferations develop in scattered island-like masses, but they soon become confluent, and large areas of pulmonary tissue assume an adenomatous appearance.

The first indication of unregulated growth consists in the enlargement and multiplication of cells bordering a portion of the lumen of the alveolus. The cells may either assume a papillomatous arrangement, or they may remain attached to the alveolar wall over a large area. The cells are of alveolar origin and are not mere enlargement of the usual cubical cells situated at the point of termination of a bronchiole.

Referring to a group of cells shown in one of the illustrations the author states that "the adenoma-like cells are characteristic of the older lesions of jaagsiekte."

While the hypertrophied cells remain in a single layer which has not yet completely encircled the lumen of the alveoli, a definite emigration of polymorphoneuclear leucocytes sets in. They occasionally penetrate into the cytoplasm of the epithelial cells, and many of them are phagocytosed by the macrophages.

At a certain stage the proliferations are said to resemble closely those described by Sir John M'Fadyean in verminous pneumonia of sheep. Indeed, Dr. Cowdry found it would be difficult to distinguish a specimen of that disease which was sent to him from jaagsiekte except by a consideration of its history.

It is in hyperæmic areas with inter-alveolar infiltrations of macrophages, some of which penetrate into the alveolar lumina, that the earliest

epithelial proliferations which are so characteristic of jaagsiekte commence.

Dr. Cowdry thought it possible that the consistent failure of investigators to transmit jaagsiekte by the direct inoculation or inhalation of diseased tissues into presumably healthy animals might indicate the operation of some predisposing factor or factors in the naturally occurring disease. Some colour was lent to this hypothesis by the observation that the lungs of a flock of sheep in which jaagsiekte was endemic suffered more than those of an equal number of sheep from a region in which the disease did not exist. The difference appeared to be significant. They centred in the interalveolar tissue, which, in about 33 per cent. of the animals was definitely thickened beyond the range of variation in this direction noted in American sheep. The thickenings occur in localised areas several millimeters in diameter. They are caused by the engorgement of the alveolar capillaries and by the accumulation of macrophages and of lymphocytes. Many of the macrophages pass into the alveolar lumina, where they assume the appearance of typical epithelioid cells. It was found that these infiltrative and exudative changes were primary to the proliferation of epithelium which always arise in parts of the lungs thus modified. It was thought that the observations of M'Fadyean, Hofman, and Eber might indicate that the pulmonary epithelium of sheep is peculiarly unstable, in other words, that it is unusually susceptible to growth stimuli or the influence tending to invalidate normal inhibitory mechanisms.

It was thought the co-existence in the same lung of many stages of reaction and areas of acute pneumonia, characterised by the accumulation and exudation of polymorphonuclear leucocytes points to the possibility that the lesions of jaagsiekte might result from a complex of influence acting together or consecutively over a considerable period of time.

In the summary it is stated that the epithelial proliferations which are so typical of jaagsiekte are chiefly of alveolar origin. They appear in areas of infiltration and exudation identical histologically with those occurring in sheep which are to all appearances normal, but which are derived from flocks in which the disease is endemic. Such lesions are said to be found in the lungs of sheep that are apparently normal, but living in flocks in which the disease is endemic.

These centres are said to become very extensive and quite atypical in structure, but never give rise to matastasis even in the adjacent lymph glands. The primary infiltration of the interalveolar tissue continues and may lead to fibrosis and extensive consolidation, or leucocytes may accumulate in large numbers, penetrate into the alveoli, and form areas of acute pneumonia. In fairly advanced cases of jaagsiekte it is common to find many stages of the reaction in one and the same lung. While it is possible that the disease may be due to a single and specific virus, and the epithelial tissues are certainly of a very special character, the pathological complex as a whole is such as might be caused by a variety of predisposing and exciting factors acting over a considerable period of time.

In 1927 Cowdry and Hadleigh Marsh published a paper* giving the result of recent studies in the north-western United States of a disease there called " progressive pneumonia," and which the authors thought had a very definite bearing upon the conception of South

* From the Laboratories of the Rockefeller Institute for Medical Research, New York, and the Montana Live Stock Sanitary Board, Helena, November 29th, 1926.

African jaagsiekte, and *vice versa*. The two conditions were studied independently by the authors, the investigator in each case considering the disease as unique and peculiar to his own geographical locality. Comparison of publications on the two diseases had brought to the attention of the authors the fact that the two chronic pneumonias of sheep occurring in widely separated parts of the world were very similar and apparently identical in the fundamental characteristics which differentiate them from other described forms of pneumonia.

"Progressive pneumonia" of sheep had been studied in Montana since 1915. It is said that the condition had been known for the last 30 years, but that it had increased in recent years and invaded new territory. It was apparently a disease of range sheep as opposed to farm flocks. It was locally believed that the disease was infectious and that the infection was probably secondary to mechanical causes. The lesions were similar to those of jaagseikte and the prospect of recovery was hopeless.

The material on which the paper was based consisted of tissues collected in South Africa from 33 sheep suffering from jaagsiekte and from 72 control healthy sheep (including some American sheep employed as geographical controls). In addition many clinical cases of jaagseikte were observed.

The progressive pneumonia material consisted of 46 cases from 18 supposedly normal sheep. Both histological and bacteriological examinations were made in "progressive pneumonia" and the symptoms were noted in many typical cases.

Here again it is said that the primary changes in the Montana cases centred in the interalveolar tissue, which was thickened. They are identical in quality, but seem to be rather more extensive in "progressive pneumonia."

The alveolar exudate is said to consist chiefly of mononuclear cells which are like those occurring in the interalveolar tissue. Their appearance in the American cases was the same as in jaagsiekte.

In a summary the authors state that as long as the exact ætiology of jaagsiekte and of "progressive pneumonia" remains unknown it cannot be said that they are identical, although on the basis of the observations described in the article they certainly appear to be. It is extremely doubtful whether it is possible to distinguish between them.

The article has three plates containing twelve good microphotographs. In the opinion of the present author these establish the fact that the disease in the United States (Montana) is identical with jaagsiekte in South Africa. In his opinion there is nowhere produced in the microphotographs anything to support the view that the lesions partake of the nature of a neoplasm.

Dr. G. de Kock, in a report published 1929, expressed the opinion that the proliferations described by Cowdry were undoubtedly of the nature of a neoplasm. "This was most characteristically borne out in three sheep with definite jaagsiekte proliferations, *i.e.*, adenomata, which caused no sign of ill-health and in which there was no complication of pneumonia."

In the material examination by him the first indication of an alteration in the very earliest lesions is the linear increase of cubical cells on the wall of the alveolus. This leads to the bulging of the alveolus into the lumen and papilliform formation. From these multiplying cubical epithelial cells which can easily be picked out in a section, from then onwards all gradations can be identified to well-formed papillomatous formations (*sic*).

The author here raises the question whether the cubical cells formed are of the nature of a metaplasia of flattened alveolar epithelium or a multiplication of pre-existing cubical epithelium, and there follows references to the histology of the lung.

The author agrees with Cowdry in the opinion that the bronchiolar epithelium contributes to the formation of the adenoma-like formations which may sometimes assume large dimensions. It appeared to him that the majority of the proliferations arise from alveolar epithelium. These papilliform formations grow and fill up the space made up of alveolar passage, atria, and alveoli, and in this way multiple circumscribed nodules are set up, each assumes a more or less regular circumscribed appearance, developing and growing into a lobule of the lung. In the early cases the adenomata in various stages of development could be identified. They may become confluent and in this way form larger nodules. The lung tissue becomes so changed by these developing adenomata that it almost resembles mammary gland adenoma.

The author's conclusions were as follows: —

1. Local congestion of the alveolar capillaries as a stage in the formation of the primary lesions of jaagsiekte could not be confirmed.
2. Papilliform "proliferations" were identified in the very early lesions without the presence of inter-alveolar infiltrations.
3. The majority of the papilliform proliferations arise from alveolar epithelium, but bronchiolar epithelium also contributes to the formation of papillomatous nodules.
4. In view of the structure of these papilliform proliferations and their growth without hope of regression, these growths are most probably true neoplasms complicated by a terminal pneumonia.
5. These neoplasms in jaagsiekte are probably of the nature of multiple papilliform cyst-adenomata.
6. All gradations of developing adenomata were studied. The lung tissue often becomes so changed by these proliferations that it resembles a mammary gland adenoma very closely.
7. The jaagsiekte foci of the nature of pearly-white circumscribed nodules, together with the pneumonia and fibrosis, formed firm, dense, very resistant, greyish-white consolidations, which may involve the greater part of the lung.
8. Above the consolidated parts of the lung, isolated, well circumscribed jaagsiekte adenomata could always be recognised.

The author thought is possible that in South Africa there might be two specific lung diseases of sheep, namely: —

(*a*) A chronic indurative catarrhal pneumonia with lymphoid hyperplasia, or infitrations, and atypical epithelial proliferations, and

(*b*) Multiple papilliform cyst-adenoma complicated by a terminal pneumonia.

De Kock's paper is accompanied by 15 microphotographs. The magnification is in most cases under low powers (under 50), but several at the end are × 100.

In the opinion of the present author they do not support Dr. de Kock's conclusions.

In the preceding Abstracts reference has been made to two articles published by the present author, and the connection which these have with jaagsiekte requires explanation.

The first was published in 1894, under the head of Verminous Pneumonia in Sheep, and it was mainly concerned with the nematode worms commonly found in the lungs of sheep, but it also referred to the minute structure of some of the lesions encountered in that study.

It has a lithographic plate with six separate photographs of sections of lungs, and five of them show embryo worms or eggs. The remaining Figure, numbered 2, shows neither, and it is the only one that has any interest in the present connection. It shows a type of pneumonia closely resembling illustrations of jaagsiekte, but at that date the name and the disease were unknown and, at least in Europe, remained unknown until Robertson's paper was published in 1904. It can now be identified as a case of jaagsiekte.

The second paper was headed Transformation of the Alveolar Epithelium in Verminous Pneumonia in the Sheep. It was published in the *Journal of Comparative Pathology and Therapeutics* in March, 1920. It dealt with lesions found in a number of sheep which actually died from pneumonia or were killed at an advanced stage of illness. The lesions involved large areas of lung which had a dirty-white colour and a somewhat fleshy consistence. Such areas were not very sharply defined but shaded off into the still spongy lung tissue. In most of the lungs examined at that time lesions of chronic pleurisy, with evidence of extensive adhesions to the chest wall, were present. No worms were discoverable in the affected portions of the lungs, but numerous eggs, embryos, and fragments of adult worms were present in the adjacent parts. It was immediately recognised that the lesions in these sheep were of very unusual character, and the paper was accompanied by nine microphotographs.

This article later attracted the attention of Dr. Cowdry in the United States, with the result that he suggested an exchange of sections with the present author. As previously noted, Dr. Cowdry found that he could not with certainty have distinguished the lesions in the English sheep from those of jaagsiekte in South Africa.

There, therefore, appears to be no doubt that the disease existed in England in 1920, and there is no ground for hoping that it has since disappeared.

Having also had an opportunity to examine sections from the diseased lungs of sheep in Iceland the present author found that the lesions could not be distinguished from those of jaagsiekte.

Aetiology.

Any alleged cause of jaagsiekte must be consistent with admitted facts concerning the disease, including the generally accepted belief that it is spread naturally by contagion. That by itself makes the view that the disease is caused by the growth of adenomatous tumours in the lungs incredible.

In the author's opinion, however, the lesions in the lungs in jaagsiekte do not resemble any form of adenoma hitherto encountered in animals or man. But even if they were of that nature they could not be used to explain the transmission of the disease.

It is more reasonable to assume that the lesions are not neoplasms but inflammatory new growths, which slowly but steadily render the respiratory epithelium functionless, and thus prove fatal.

In other words, it appears to be most probable that the cause of the disease is a virus which multiplies only in the lungs and escapes by the air passages, to cause fresh infections in the flock.

Conclusions.

The cause of the disease has not been determined with certainty.

It is not caused by an adenoma or other neoplasm.

The name jaagsiekte should be retained. It has the double advantage that it is already in general use, and does not connote any theory regarding the cause of the disease.

The lesions are pathognomonic both in respect of their appearance and their histology.

The disease has been identified in South Africa, England, and Iceland, and the disease of the sheep in Montana in the United States is probably of the same nature.

Further researches, especially regarding possible methods of infection, are urgently necessary.

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CONTENTS

GENERAL ARTICLES.

PAGE

Enzootic Pneumonia in Sheep. By R. F. Montgomerie, T. J. Bosworth, and R. E. Glover 87

Equine Contagious Pneumonia. German *Brustseuche.* By Sir John M'Fadyean... 108

Studies in Animal Trypanosomiases. IV.—The Effect of *Trypanosoma congolense* and *Trypanosoma brucei* on some Inorganic Blood Constituents. By M. H. French ... 119

ABSTRACTS.

Fowl Leukosis 128

Pseudo-Tuberculosis of Rodents 139

Experimental Researches regarding Aujeszky's Disease or Pseudo-Rabies 143

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